OBSERVATIONS ON THE MYCORRHIZAS OF FOREST TREES

II.* THE RHIZOSPHERE OF *PINUS RADIATA* D. DON

By R. C. Foster† and G. C. Marks‡

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Summary

Two common mycorrhiza types of *Pinus radiata* were examined by light and electron microscopy. Large numbers of bacteria and fungal species other than those forming the mycorrhiza as well as diatoms were observed in the mycorrhizosphere. Different morphological types of bacteria were characteristic of different mycorrhizal types, and in some cases the bacteria were associated with lysed regions of the mantle. The distribution of the bacteria within the rhizosphere is discussed in relation to the clay minerals and the carbohydrate and polyphenol metabolisms of the host.

Within the mantle and the Hartig net, the hyphae have a structure typical of basidiomycetes, but within the tannin layer the hyphae have an abnormal distorted appearance. This is correlated with changes in cytology and is construed as evidence of the production of polyphenols toxic to fungi. A possible role of these polyphenols in the control of mycorrhizal associations by selective action on soil fungi entering the rhizosphere is discussed.

I. Introduction

Although a few direct observations have been made on the rhizosphere of seedlings (Starkey 1938; Lindford 1942; Jenny and Grossenbacher 1962; Dart and Mercer 1964) there are none reported on the ectotrophic mycorrhizosphere of *Pinus radiata* D. Don, the most widely used softwood in south-eastern Australia.

If Hiltner's (1904) definition of the rhizosphere is accepted there is no difficulty in deciding its limits in ectotrophic mycorrhizas. In contrast the limits of the rhizoplane (Clark 1949) are difficult to define in a mycorrhiza. Part of the problem lies in the assumed role of the mycorrhiza fungus. We consider it to be a root-inhabitating fungus (Garrett 1956) which forms a close association with the host in the Hartig net, i.e. in the epidermis and outer cortex of the root. In Part I of this series (Foster and Marks 1966) we showed how intimate the relation between the host and fungus is. It was evident that the walls of the inner host cells are separated from one another by the fungal hyphae, and we consider that the rhizoplane occurs at the boundary between the host and the fungus, in which case the effective area of the rhizoplane extends to the limits of the Hartig net region and is increased considerably over that of the unpenetrated root. The object of the present paper is to describe the fine structure of the surface of the fungal and host cells and of the other components of the rhizosphere.

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† Division of Forest Products, CSIRO, Melbourne.

‡ Victorian Forests Commission, Melbourne.

II. Materials and Methods

Mycorrhizal roots were obtained from both young seedlings and mature 40-year-old *P. radiata* trees growing at Mt. Macedon, Vic. Three common mycorrhiza subtypes (Dominik 1959) were extracted from the forest soil, using a wet-sieving technique (Marks 1965). These were a white form (subtype B, genus Ba), a red form (subtype F, genus Ff), and a pseudomycorrhizal black form (subtype F, genus Fh). In the case of seedling material, the finer lateral roots were washed in tap water to remove the bulk of the adhering soil and the mycorrhizal rootlets dissected away. Three types of preparation were made for electron microscopy in addition to that previously described (Foster and Marks 1966). One group of short roots were replicated in carbon by a wet-replica technique to reveal the surface structures of the rhizosphere components. A second group was gently teased in water with fine needles to separate individual hyphae from the mantle. These were mounted on electron microscope grids for direct observation. A third group was gently macerated in 2% HCl followed by 2% NaOH and suspensions of the components placed on grids after suitable dilution, dried down, and shadowed. The specimens were examined in a Siemens electron microscope at 60 kV.

III. Results

Marks (1965) has described seven distinct morphological mycorrhiza forms on *P. radiata* growing at this forest site. These are shown in Plate 1, Figure 1, and consist of a red (*Ff*), a reddish grey (*Bb*), a cottony white rhizomorphic (*Ca*), a blue-green, a creamy white (*Ba*), and two black types (*Fh*) and (*Ka*), both of which are thought to be parasitic. Attention is confined to *Ba*, *Ff*, *Kh*, and *Fh* (indicated by the arrows).

These mycorrhizas differed markedly in colour and texture and are therefore readily distinguished.

The various types of short roots were distinctive in transverse sections. The white type *Ba* (Plate 1, Fig. 2) had narrow felt-like hyphae orientated transversely with respect to the axis of the short root, whilst the red type *Ff* (Plate 1, Fig. 3) had wider hyphae with a parenchymatous appearance so that the hyphal segments appear shorter in section. Both types have a well-developed tannin layer (*T*) consisting of one or two layers of host cells filled with polyphenolic materials. The fungal hyphae pass between these tannin cells into the Hartig net region (*H*). The white type frequently had polyphenolic compounds in the mantle (arrowed) as described previously (Foster and Marks 1966).

Electron micrographs of carbon replicas of washed, short, mycorrhizal roots show the surface to be covered with matted hyphae. Usually only one type of hypha was present. In the white (*Ba*) type (Plate 2, Fig. 1) they were loosely woven, passing for several microns over the root before passing under another hypha. In the red (*Ff*) type, however, they were densely interwoven, so that only short lengths were exposed (Plate 2, Fig. 2), a feature in keeping with its pseudoparenchymatous structure.

The various mycorrhiza types were not only distinguishable by their gross morphology but also by differences in diameter and appearance of the hyphal walls in the mantle. The sculpturing seen in many of the fungi may not provide a significant taxonomic criterion because of its variability along the length of a single hypha.

Usually the fungi with sculptured walls had a larger diameter than the smooth-surfaced types (Plate 2, Fig. 4). The sculpturing was sometimes predominantly angular with hexagonal outlines in some samples (Plate 2, Fig. 5) but more irregular in shape (Plate 2, Fig. 3) in others.

Bacteria were closely associated with the mycorrhiza mantle. In carbon replicas they appeared as ovoid bodies lying on the surface of the hyphae (Plate 2, Fig. 1, circle) or in the soil adjacent to them (Plate 2, Fig. 4, Plate 3, Fig. 1, arrows). In some morphological features the bacterial population of the black mycorrhizas differed from that of the red and white types. Those associated with the white (*Ba*) and red (*Ff*) types of mycorrhizae were usually ovoid (3 by 0·6 μ) and occurred either singly (Plate 3, Fig. 2) or in groups (Plate 3, Fig. 1). Similar ovoid bacteria were occasionally associated with the black type of mycorrhiza (*Fh*) but the bacterial type seen most frequently was an elongated (5 by 0·5 μ) species (Plate 3, Fig. 4). Occasionally peculiar filiform structures of unknown origin could be observed in the vicinity of the black type. It may be concluded that, in *P. radiata*, the bacterial population in the mycorrhizosphere depends on the type of mycorrhiza formed. In glutaraldehyde- and osmium-fixed material the bacteria showed a thick electron-dense wall with fine radiating filaments (Plate 3, Fig. 3) which may represent denatured mucilages. The cytoplasm was typical of bacteria.

Parts of the outermost layer of hyphal cells in the mantle sometimes appeared to have lysed, forming gaps containing osmiophilic substances (Plate 3, Fig. 5, arrow). These gaps were filled with bacteria, apparently multiplying in the breakdown products of the hyphae. In some cases bacteria were seen deep within the mantle (Plate 5, Fig. 1, arrow). In these situations they were always confined to the interhyphal spaces and there was no indication of entry into either the hyphae or the host. At higher magnifications (Plate 5, Fig. 2) these bacteria appeared to be identical to those in the soil and at the surface of the root. The spatial distribution of the bacteria in the mycorrhizosphere is shown in a section through a red (*Ff*) type of association (Plate 4). The uneven distribution of bacteria is immediately apparent. At the upper right is the tannin layer of the host (*T*). The mantle (*M*) lies below this. The hyphae next to the tannin layer are rich in cytoplasm while those adjacent to the soil are devoid of cytoplasmic contents. The soil (*S*) is filled with electron-dense bodies of various types and between them numerous bacteria (arrowed) are found. In this plate over 50 are seen in the small area photographed. Most of them lie in a definite band 0–12 μ from the surface of the mantle.

An attempt was made to determine the actual numbers of bacteria in the various layers of the mantle and the mycorrhizosphere. The results (Table 1) show that the largest bacterial populations exist in the outermost mantle layers and those areas of the soil colonized by fungal hyphae that may have originated from the mantle. The hyphae in this zone appear senescent, with few organelles and no visible glycogen. It is possible that the fungal cells support the greatly increased bacterial population in this region. It is noteworthy that the bacterial population in the outer layers of the mantle is about 16 times that found in the outermost region of the mycorrhizosphere and that no bacteria could be detected close to the tannin layer.

Cross sections of the mycorrhizospheres (Plate 4) show two types of electron-dense objects. The first comprised non-crystalline, irregular, osmiophilic masses,

presumably humus, and the second were regular crystalline masses consisting of soil minerals. The latter usually fragment on cutting for electron microscopy but sufficient numbers of particles remained *in situ* to give electron-diffraction patterns which helped establish their identity. In the replicas hyphae were frequently seen to be overlying crystalline bodies like sand grains. No preferential associations were formed between any fungal or bacterial species and a particular mineral type as suggested by Stotzky and Ren (1966).

TABLE 1

DISTRIBUTION OF BACTERIA IN THE RHIZOSPHERE OF A TYPE *Ff* MYCORRHIZA OF *P. RADIATA*

Distance from Tannin Layer (μ)	Appearance and Texture of Mantle	Glycogen Content of Fungal Hypha	No. of Bacteria per cm^3
0-4·0	Closely packed, impregnated with polyphenols	Glycogen densely aggregated and nearly contiguous in cells	0
4·0–8·0	Loose, felt-like	Large spaces appear between aggregations	144×10^9
8·0–12·0	Loose hyphae	Few aggregations in each cell	222×10^9
12·0–16·0	Hyphae mixed with soil particles	No visible glycogen	111×10^9
16·0	Soil particles only	No visible glycogen	14×10^9

Occasionally small objects with regular exterior sculpturing were found in the soil adjacent to the hyphae. These may be small diatoms (Plate 3, Fig. 4, arrowhead).

Whole mounts of hyphae from the mantle region revealed loose-branched hyphae about 4 μ in diameter (Plate 5, Fig. 3) with typical clamp connections (Plate 5, Fig. 4, arrows). The hyphal surface appears amorphous but in macerated material where some of the matrix substances of the wall are extracted an irregular network of microfibrils was revealed (Plate 5, Fig. 5). There was no sign of a crossed structure or of a marked change in microfibrillar orientation in the clamp connections. The texture of the wall was close and no pores or patches with more open pit-like arrangements were observed. In the region of the tannin layer the hyphae showed masses of wide, considerably distorted hyphae (Plate 6, Figs. 1 and 2). They were covered with short protrusions (white arrows) which had a normal microfibrillar arrangement at the surface. The protrusions were not cut off from the parent hypha by septae. The hyphae themselves, however, had frequent septae (Plate 6, Fig. 1; see also Fig. 2, arrows) which could be shown to be dolipore septae as follows. Plate 8, Figure 6, shows that dolipore septae are present in the tannin region of the mantle. In longitudinal section (Plate 8, Fig. 5) the pore is shown to lie in the septal swelling which in tranverse section (Plate 8, Fig. 4) shows an annular outline. When the tissues are macerated to obtain hyphae from this region most of the substance of the septal swelling is extracted, but sufficient remains to give an electron-opaque annulus

round the pore (Plate 6, Fig. 1, black arrow). This is more obvious in Plate 6, Figure 3, where the surface hyphae, which are distinct at the periphery of the septum, are obscure near the pore. In the region of the Hartig net the host cells were large, cubical in shape, and covered with a branching hyphal network (Plate 7, Fig. 1). The hyphae were similar in shape and branching characteristics to those of the mantle although clamp connections were rare. They were septate (Plate 7, Figs. 1 and 3) with conspicuous septal plates (Plate 7, Fig. 4) which have been shown to contain dolipores.

Although the microfibrils of the host walls were observed clearly in macerated material, those of the fungal hyphae were not (Plate 7, Fig. 4). The host microfibrils were distinctly orientated and since the penetration path of the fungus occurs along the middle lamella (Plate 8, Fig. 3) this orientated layer must form the outermost microfibrillar layer of the wall. There was no sign of lysis of the cellulose fraction of the host wall. Hyphae penetrating between the host cells frequently appeared wedge-shaped in section (Plate 8, Fig. 3) and had small protuberances along the leading edge. Amongst the hyphae associated with the Hartig net cells, which could be readily distinguished from other host cells by their large size, there were hyphae with small, irregularly lobed tips (Plate 8, Fig. 2). Sections through the Hartig net showed that during the initial stages of penetration the hyphae were narrow but later they became more rounded (Foster and Marks 1966) and such hyphae with considerably swollen tips were occasionally encountered in Hartig net macerations (Plate 8, Fig. 1). It is thought that this change in size causes the splitting of the middle lamellar substance (Plate 7, Fig. 2).

IV. Discussion

Some of the problems of classifying the mycorrhizas of *P. radiata* have been discussed elsewhere (Marks 1965). Two important difficulties were noticed in this work. One consisted in determining whether the mycorrhizas examined were representative of the area and the other was ascertaining whether a particular type of mycorrhiza was formed by more than one fungus.

Earlier surveys of the site had established that the types of mycorrhiza reported on were species of a stable form, the red and white forms being the most widely distributed and frequently observed over a period of three years. Thus there is little doubt that the types examined are representative of the area.

One of the difficulties in determining whether more than one fungus can form the same type of mycorrhiza is that the morphology of the fungus varies with nutritional status. At a fixed distance behind the root apex, however, it is possible that the age and nutrition of a fungus component will be reasonably uniform, thus making it possible to identify the fungal component on the basis of its cytology. Comparative studies of the fine structure of different mycorrhiza collections from *P. radiata* and *Pseudotsuga menziesii* (Foster and Marks, unpublished data) showed that there was consistency within the mycorrhizal types of the two species so that it was possible to identify these mycorrhizal types by the cytology alone. On the basis of these observations we believe that a single fungal species will form a mycorrhiza with constant characteristics in a given soil environment.

Several authors (e.g. Hawker 1965) have noted that few studies of the surface of fungi are reported in the literature. As far as we are aware no details of surface sculpturing of fungal hyphae have been published. The accretion of various crystalline materials such as calcium oxalate and the secretion of pigments has been observed in basidiomycetes (Nobles 1948; Cunningham 1963). Some of the protuberances seen in the electron microphotographs are distinctly hexagonal and are of the same order of size as those described in the Thelephoracae by Cunningham (1963). Hacskaylo (1965) reports that members of this family are mycorrhiza formers. If these are true crystals their function is unknown but in all probability they are merely waste products. The identity of the fungal component of the white and red types is not known.

It is known that the outer surface of fungal hyphae are covered with an amorphous layer (Aronson and Preston 1960; Parker, Preston, and Fogg 1963; Hawker 1965), and that below this there is a microfibrillar layer of chitin or cellulose (Hawker 1965), or both (Fuller and Barshad 1960). Aronson and Preston described the wall in certain Phycomyces as having randomly arranged and intertwining microfibrils at the surface, as in the present case. In some species a cross structure was observed (Aronson and Preston 1960). No trace of this was seen in the present material, nor were there any of the disturbed areas observed by Strunk (1963) in *Coriolus versicolor* that resembled primary pit fields, even in the much-distorted hyphae of the tannin region where frequent and irregular branching took place.

The formation of dolipore septae has been described for many basidiomycetous fungi (Moore and McAlear 1962; Bracker and Butler 1963) and the pore is described in surface view. It would appear that removal of the septal swelling material in the maceration process somewhat enlarges the septal pore. It will be noted that these are distinct from the micropores of *Geotrichum candidum* described by Hashimoto, Kishi, and Yoshida (1964) and Welsenach and Kerpel (1965). Although the morphology and general cytology are changed the dolipore structure remains unaltered in the Hartig net zone.

The cells in the tannin region were of particular interest. Reference has been made to their abnormal cytology (Foster and Marks 1966). The irregular branching is characteristic of hyphae growing in toxic media (Hütter *et al.* 1966). It is interesting to note that polyphenols have been shown to modify growth patterns presumably by the inhibition of enzymes and perhaps growth regulators. Polyphenols are known to be fungistatic (Offord 1940) and to inhibit cellulase (Mandels and Reese 1963) and this may account for the abnormal morphology of the hyphae within this region. Large amounts of polyphenols may be produced in the host cells and secreted into the intercellular spaces so that they partially enclose the fungal hyphae. It is significant that the hyphae in the Hartig net region, where the secretion of tannins is considerably reduced, show a normal cytology (Foster and Marks 1966).

These observations on the effects of the tannin layer are of considerable importance. It is obvious that one of the "characteristics" of a mycorrhiza fungus would be its ability to survive the toxic effects of this layer, whether it arose before or after infection. As a consequence this layer would have a selective action on soil fungi entering the root. It is possible that the polyphenolic compounds could provide an additional chemical barrier to the "biological barrier" referred to previously (Zak 1964).

Several studies of host–parasite relationships have been made (Ehrlich and Ehrlich 1963; Peyton and Bowen 1963; Foster and Marks 1966) but none of the wall surface at the host–parasite boundary. In the Hartig net the fungal hyphae were closely pressed to the host wall but there was no lysis of the cellulosic fraction of the wall. In the mycorrhizas it was obvious that only the middle lamella substance is attacked and it is significant that the microfibrils of the host walls were revealed on maceration but not those of the fungi. Hawker (1965) has drawn attention to the meagre information about the chemical composition of the various fractions of the fungal cell wall. It is known that the fungal wall contains up to 40% of hemicelluloses (70% in yeast) (Roelofson 1959), and these are probably different to those of the host wall.

The possibility was raised (Foster and Marks 1966) that penetration into the cortex of the host by the fungus is accomplished by means of a "wedging action" whereby glycogen is hydrolysed to low molecular weight moieties in the small angular protrusions from recently formed hyphae and that the resulting increase in osmotic pressure results in a mechanical force splitting the host wall along the predigested middle lamella region. The present results would give visual support to this hypothesis since the small outgrowths of the hyphal tips that could accomplish this task can be readily seen.

Numerous instances are known where the fungi enter the living cells of the host. In older degenerating mycorrhizas of *P. radiata* intracellular penetration is very common. Situations have also been described in other species where intracellular penetration occurred under conditions of poor aeration (Marks 1965). Thus, the question arises as to why the fungi do not enter the host cell in healthy mycorrhizas. There are two possible explanations of this. Firstly, the cellulases produced by the fungus may be inhibited by specific component(s) of the tannin fraction secreted by the host. If enzyme inhibitors are involved, a specific cellulase inhibitor may be present as pectinases are still able to function. Secondly it is known that cellulase is a facultative enzyme (Mandels and Reese 1963) and it is possible that as long as carbohydrate is present in an available form its synthesis is suppressed. We have shown (Foster and Marks 1966, Plate 8, Fig. 3) that hyphae within the Hartig net are full of glycogen, indicating that carbohydrate is in good supply in this region.

There is little doubt that nitrogen accretion occurs in *P. radiata* forests (Stevenson 1959), and there is sufficient evidence to indicate that the mycorrhizas are associated with this phenomenon, (Richards and Voigt 1964; Hewitt 1966). The discovery that bacterial contaminants (Richards and Voigt 1964) could influence nitrogen fixation is of particular interest in view of the enormous numbers of bacteria in the mycorrhizosphere and the fungal hyphae of the mantle. The weight of mycorrhiza in a stand of *P. radiata* is considerable. Marks and Foster (unpublished data) found that, in a 34-year-old stand growing on a class "one" site in Victoria, there was approximately 1200 lb/acre of mycorrhizas in the top 6 in. of soil. It is apparent that a close nutritional relationship exists between the two organisms. Mannitol is one of the carbohydrates used by bacteria, and Lewis and Harley (1965*a*, 1965*b*, 1965*c*) showed that beech mycorrhiza secrete this substrate. Hassouma and Wareing (1964) found that in the presence of mannitol rhizosphere bacteria could fix nitrogen. Thus it is possible that the nitrogen fixation observed on conifer stands is accomplished by

the bacteria in the mycorrhizosphere under the influence exudates from the fungal mantle. The observations of Katznelson, Rovatt, and Peterson (1962) and those reported here indicate that each mycorrhiza type can have a specific bacterial association and this may affect the efficiency with which nitrogen fixation can take place.

The intimate association between the bacteria and the lysed part of the mantle is very interesting. Some bacteria are capable of lysing fungal hyphae and in this respect the fact that many outer fungal hyphae are devoid of cytoplasm may be significant. In the present instance, however, it is impossible to determine if lysis was caused by the bacteria or whether they multiplied under the influence of the nutrients released by some other agency. The dense bacterial population in this region indicates how favourable the mycorrhizosphere environment is for bacterial growth. Zak (1964) supposed that the mycorrhiza fungus may secrete antibiotics and so form *inter alia*, a biological barrier to soil pathogens. We suggest that the bacterial shell of the mycorrhizosphere could have similar functions.

A proper understanding of the nutrition of *P. radiata* and some of the problems associated with it (Keeves 1966) can only be achieved after careful study of the soil environment of the tree. These direct observations on the rhizosphere and rhizoplane of the common forms of mycorrhizas of *P. radiata* show that complex associations are formed between the rootlet, the mycorrhiza fungus, and the fungi and bacteria in the soil. At present very little is known about the bacterial members of the association and their part in tree nutrition or their interactions with the various types of mycorrhizas formed by *P. radiata*. It is hoped that further work in this area will provide more information on the nutrition of this important tree species.

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EXPLANATION OF PLATES 1–8

The following symbols are used on Plates 1–8: *B*, bacteria; *F*, fungal hypha; *H*, Hartig net; *HC*, host cell of Hartig net; *M*, mantle region; *T*, tannin cell region; *S*, soil

PLATE 1

Fig. 1.—Common types of mycorrhizal associations found on *Pinus radiata* from Mount Macedon, Vic., showing their distinct morphology and colour. *Ff*, red type; *Bb*, reddish grey type; *Ca*, cottony white rhizomorphic form; *Ba*, creamy white type; *Fh*, "*mycelium radicis atrovirens*"; *Ka*, *Coenococcum graniforme*. ×5.

Fig. 2.—Cross-section of white type (*Ba*) showing texture of mantle.

Fig. 3.—Cross-section of red type (*Ff*) showing pseudoparenchymatous texture of mantle.

PLATE 2

Electron micrographs of carbon replicas of mycorrhizas

Fig. 1.—White type (*Ba*). The hyphae are linear and relatively unbranched, with bacteria (*B*) present. ×2700.

Fig. 2.—Red type (*Ff*), showing much-branched interweaving hyphae. ×2000.

Fig. 3.—Black type (*Fh*) showing the characteristically rough surface. ×27,000.

Fig. 4.—Black type with bacterium (arrow). ×9000.

Fig. 5.—Black type showing details of hexagonal protuberances at the surface. ×30,000.

PLATE 3

Details of the mycorrhizosphere

Fig. 1.—White type (*Ba*) fungal hypha with associated bacteria (*B*) overlying the hypha or in clusters in the adjacent soil. Carbon replica. ×6000.

Fig. 2.—White type (*Ba*) hypha with ovoid bacterium. Carbon replica. ×6000.

Fig. 3.—Section of ovoid bacterium associated with the red type (*Ff*) hyphae. ×50,000.

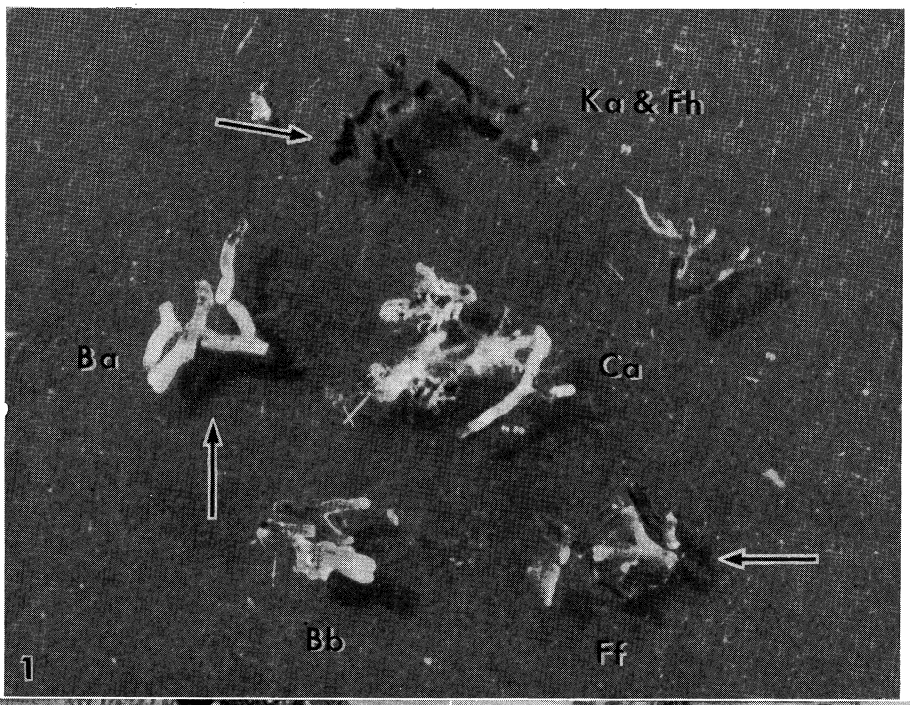
Fig. 4.—Black type hypha with elongate bacteria (arrow) and diatom (arrowhead). Carbon replica. ×6000.

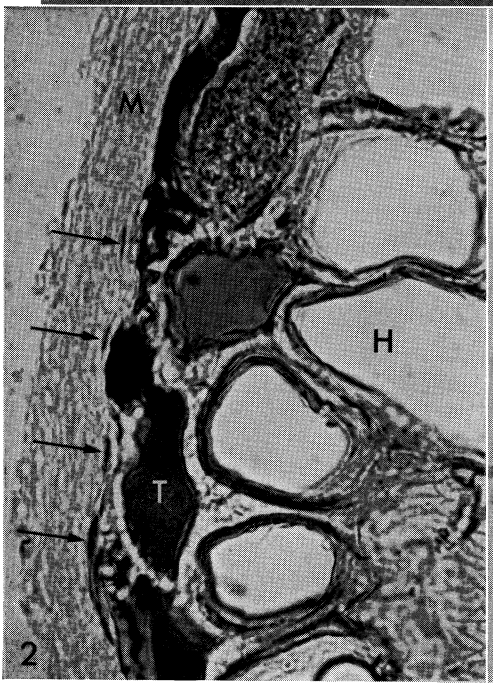
Fig. 5.—Section through red (*Ff*) association showing lysis of the mantle. There is accumulation of osmiophilic materials (arrow) in the lysis region which have attracted several bacteria (*B*). ×3000.

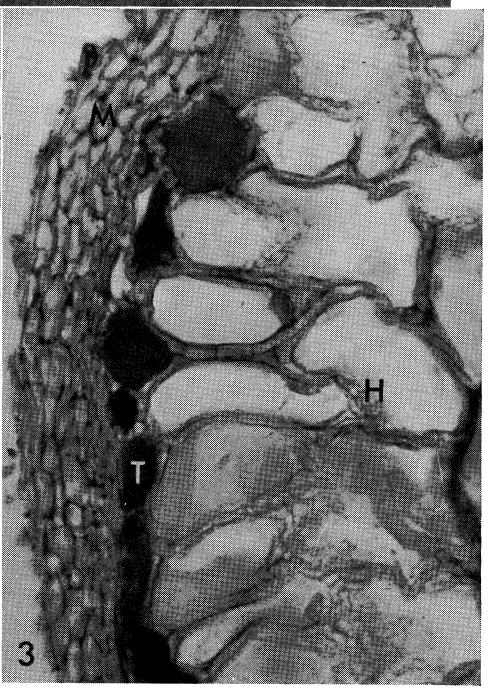
PLATE 4

Ultra-thin section through a red type association showing the tannin layer of the host (*T*); the mantle (*M*) consists of cytoplasm-filled hyphae towards the root and empty hyphae towards the soil (*S*). The soil contains irregular osmiophilic particles as well as crystalline clay minerals. Note the great abundance of bacteria (arrows). Fixed in glutaraldehyde and osmium tetroxide. ×6000.

OBSERVATIONS ON THE MYCORRHIZAS OF FOREST TREES. II







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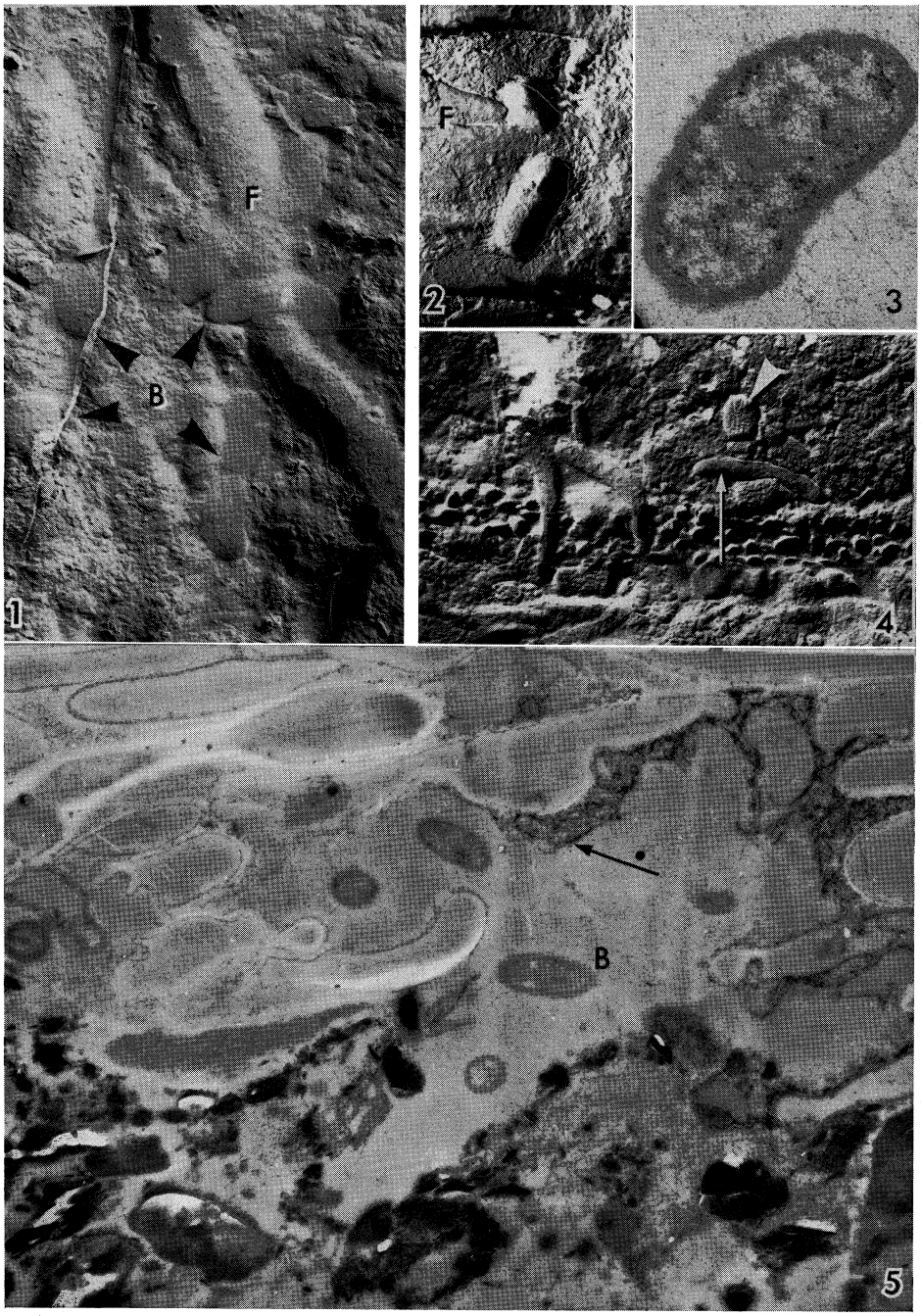

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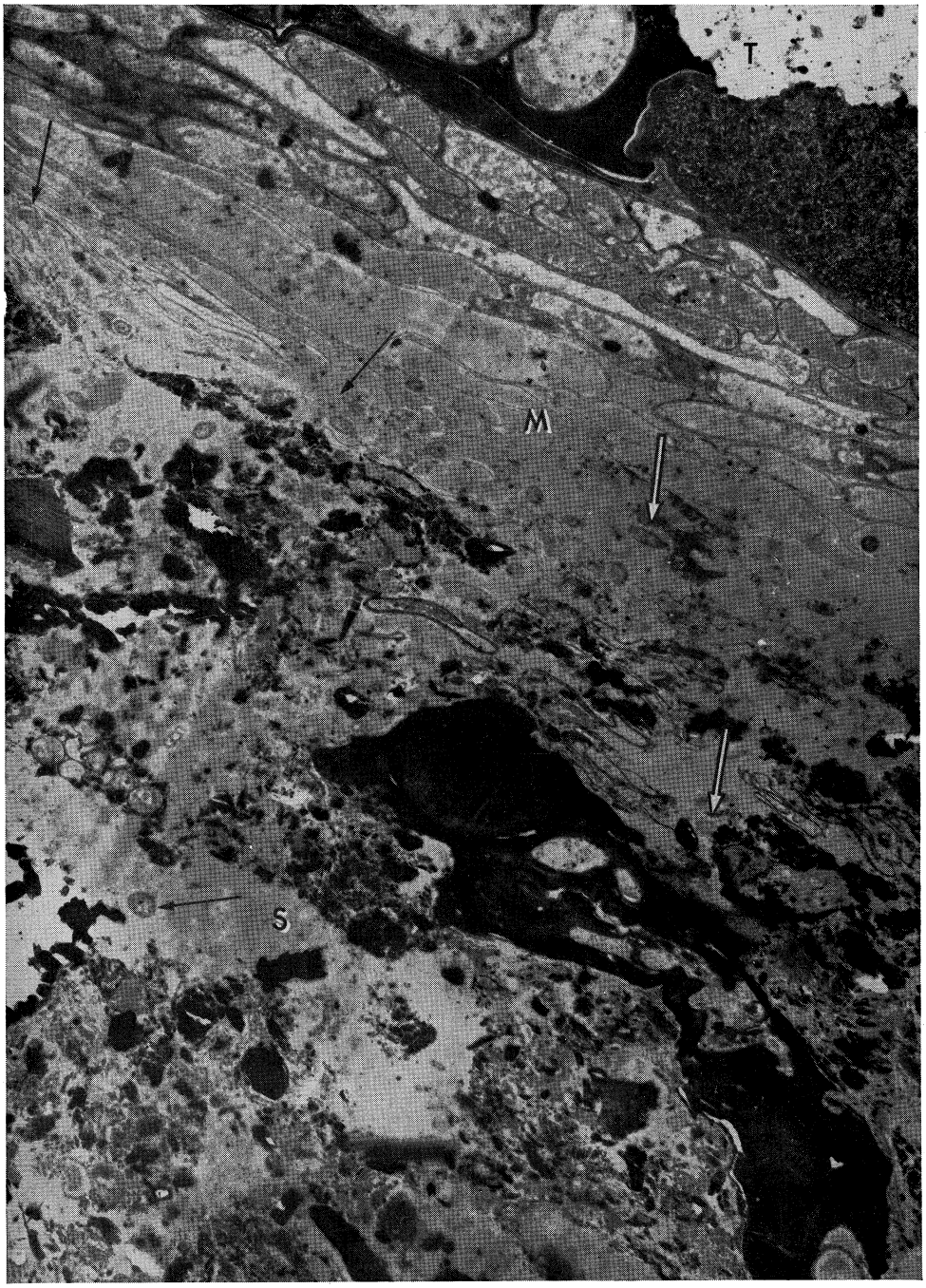

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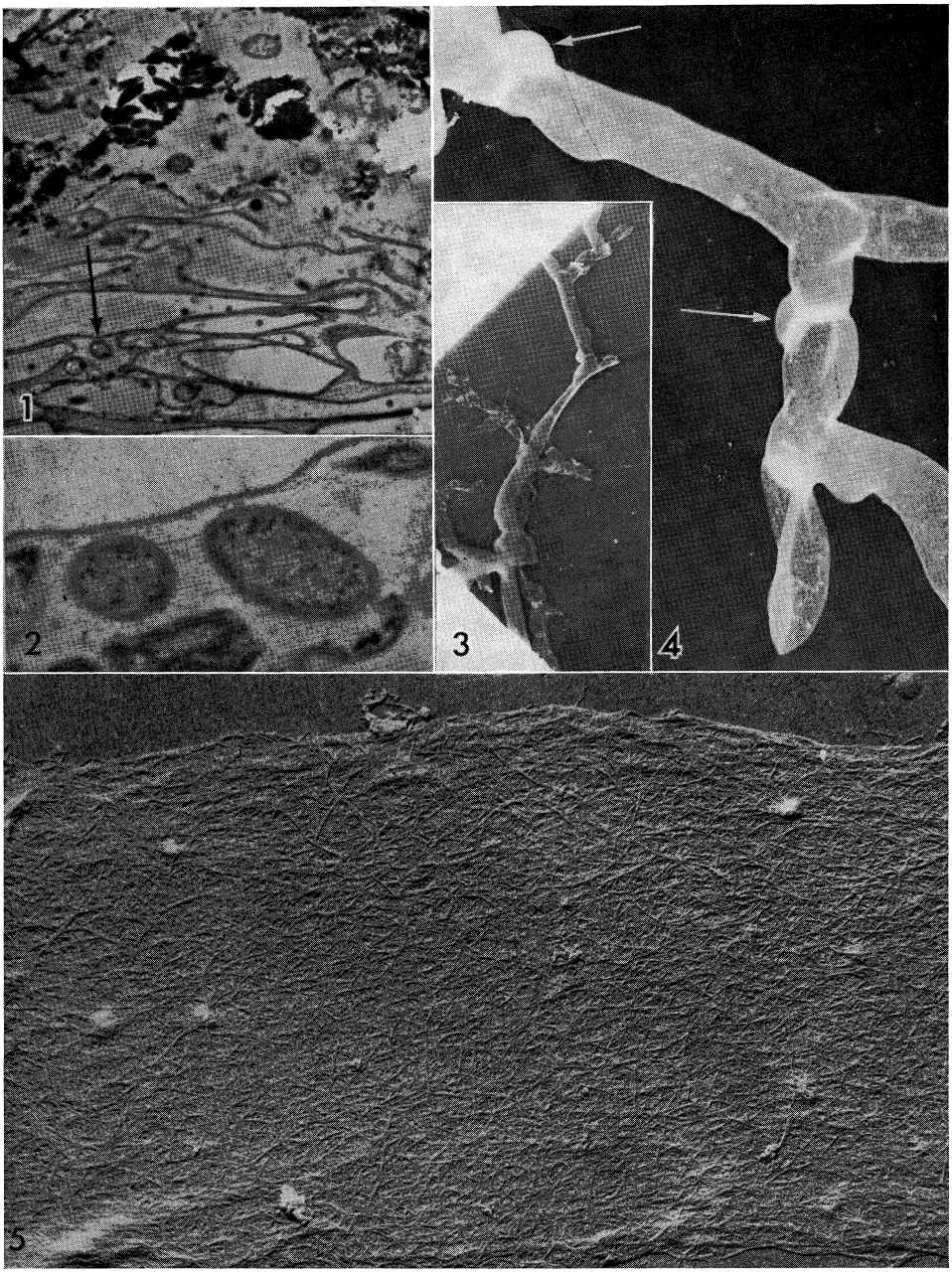

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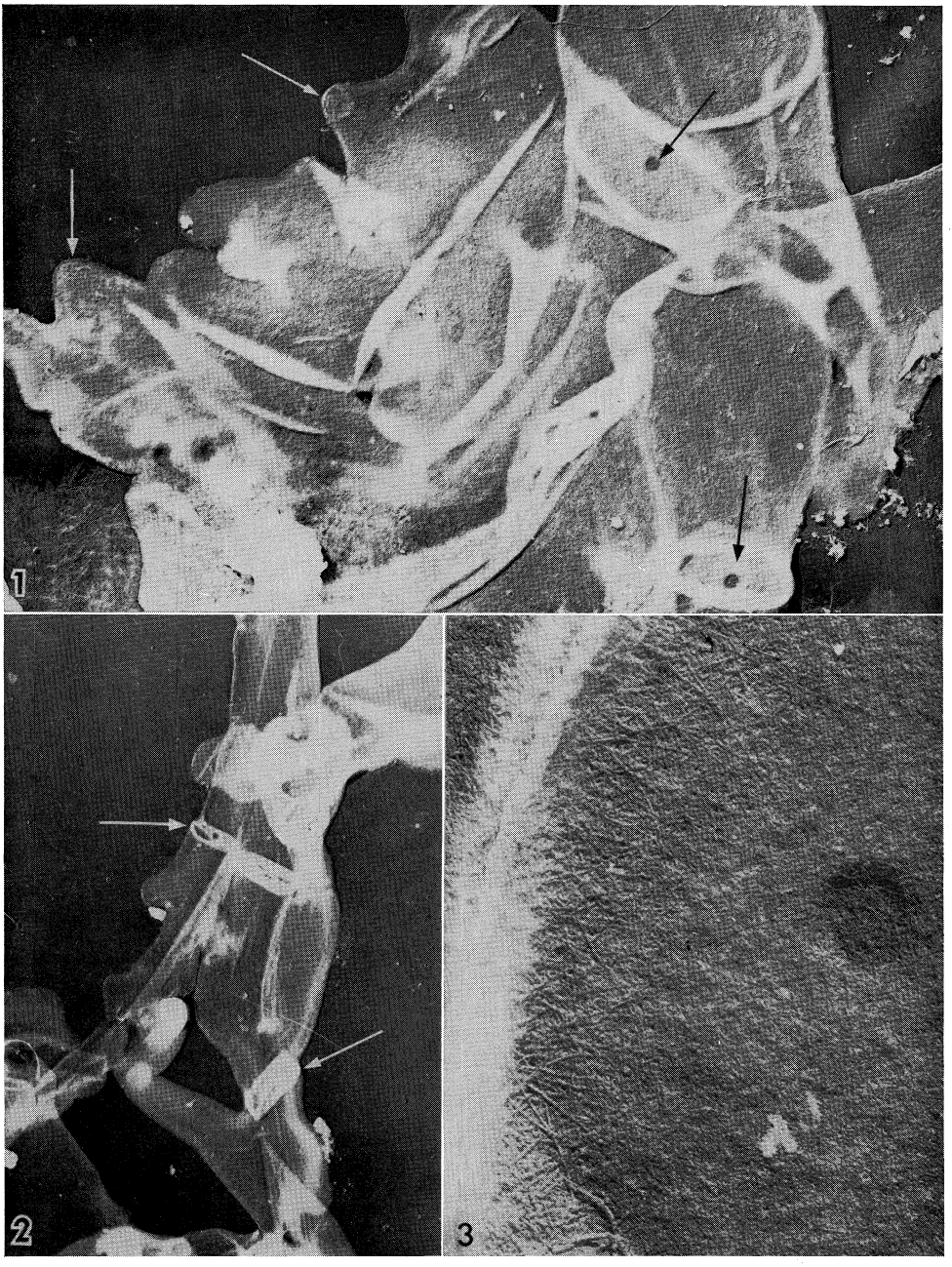

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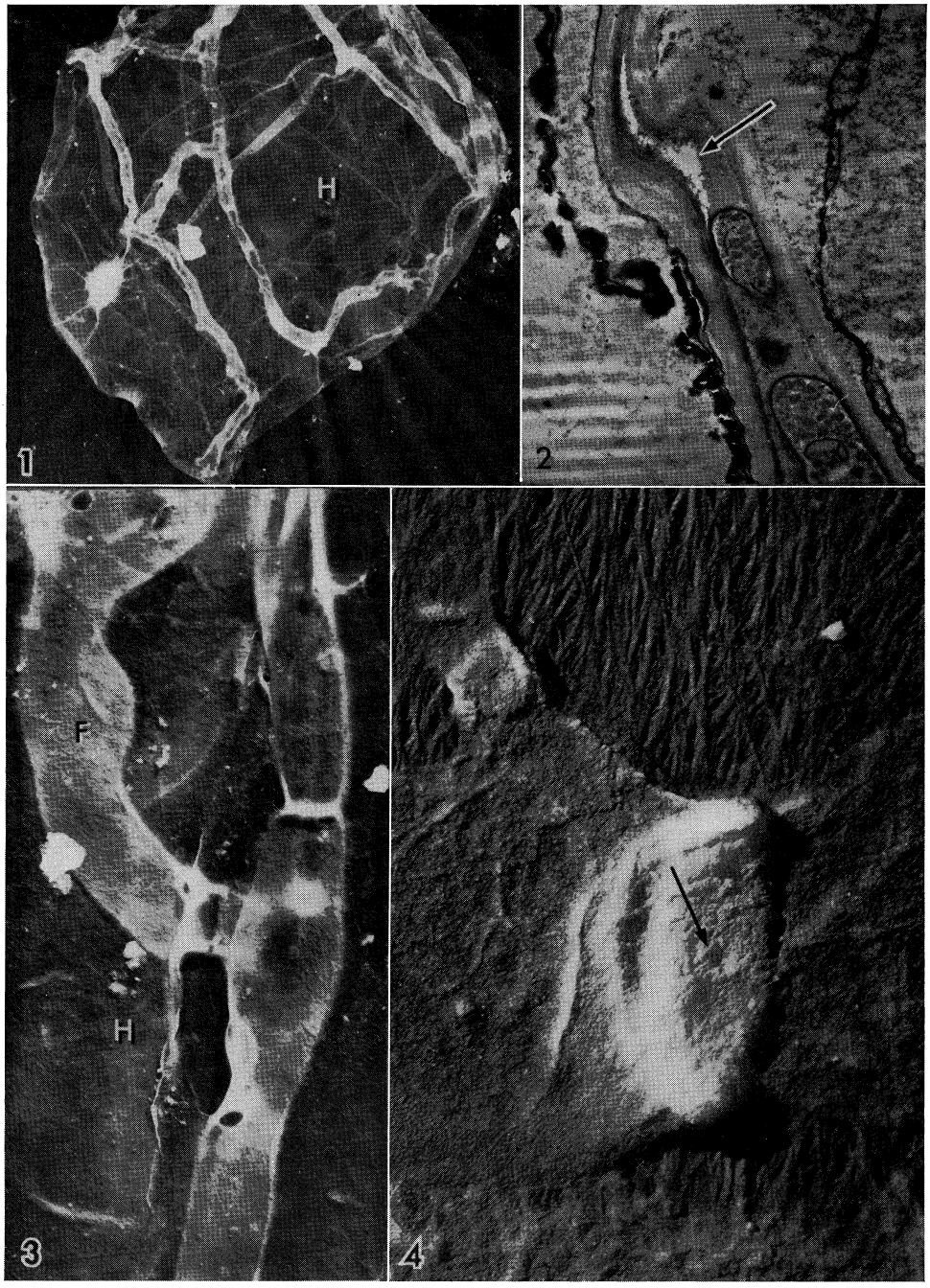

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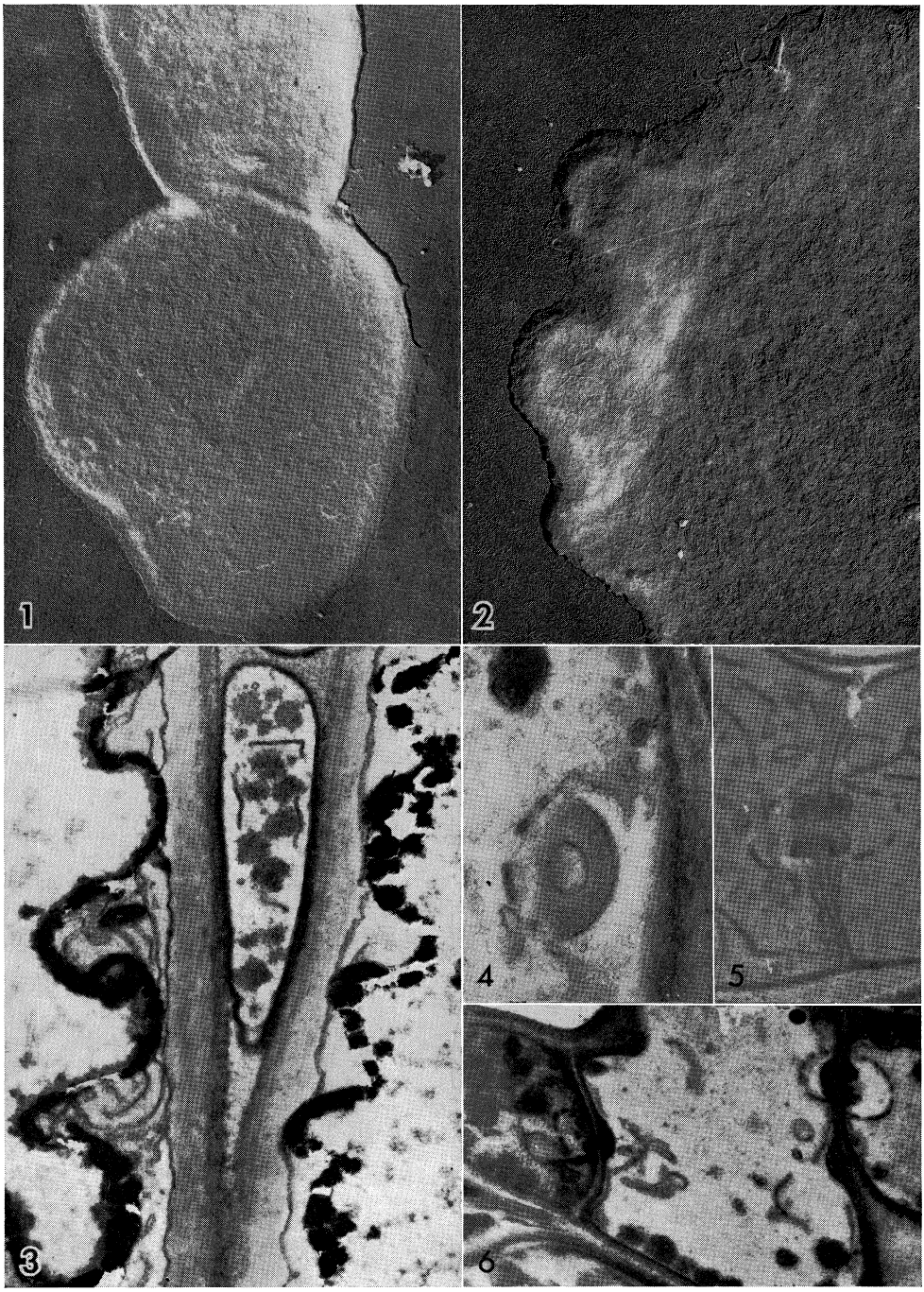

PLATE 5

Fig. 1.—Section of red type association fixed in glutaraldehyde followed by osmium tetroxide, showing bacteria within the mantle (arrow). ×5000.

Fig. 2.—Details of bacteria from within the mantle. Fixed as in Figure 1. ×50,000.

Fig. 3.—Whole-mounted mantle hyphae from a white-type mantle. ×200.

Fig. 4.—Details of Figure 3 showing clamp connections and branching hyphae. ×300.

Fig. 5.—Details of hyphal structure after chemical removal of the encrusting substances. Note chitin microfibrils with irregular orientation. ×15,000.

PLATE 6

Details of hyphae from tannin cell zone–white-type association

Fig. 1.—General view showing lobed hyphae (white arrows) and perforated septa (black arrows). ×15,000.

Fig. 2.—General view showing relatively short cells and frequent septa (arrows). ×10,000.

Fig. 3.—Details of septum showing septal pore. ×50,000.

PLATE 7

Details of hyphae from Hartig net of white-type association

Fig. 1.—Isolated host cell with attached fungal hyphae. Note normal shape, infrequent branching, and lack of septa of the hyphae. ×500.

Fig. 2.—Section of Hartig net showing glycogen-filled hyphae in middle lamellar region of host wall. Note the rounded fungal cells and the split in the middle lamellar region (arrow). Fixed in potassium permanganate. ×10,000.

Fig. 3.—Details of Figure 1 showing branching hyphae with septa. The hyphae have become flattened in preparation except at the septae, giving a false constriction. ×4000.

Fig. 4.—Detail of hypha on host wall. Note the flattened dolipore septum (arrow) and that the host wall appears extracted whilst the fungal wall does not. There is no trace of attack of the cellulose fraction of the host wall. ×30,000.

PLATE 8

Details of the Hartig net are shown in Figures 1–3; details of dolipore septa in Figures 4–6

Fig. 1.—Swollen hyphal tip which is assumed to give rise to wedging action in fungal penetration. ×20,000.

Fig. 2.—Small protuberances at tip of hyphae equivalent to those in Figure 3. Note close microfibrillar texture. ×20,000.

Fig. 3.—Small hypha penetrating between two host cells. Note small protuberances from the leading edge of the wedge-shaped fungal cell and the separation of the host wall along the middle lamella. ×10,000.

Fig. 4.—Oblique section through dolipore from the mantle region showing the septal swelling (ovoid annulus), the septal pore, lined with a lipoprotein membrane, and the parenthosome cap (perforated). ×35,000.

Fig. 5.—Dolipore from Hartig net region showing septal pore, septal swelling, and perforated parenthosomes. Note that all but the septum is extracted in Figures 5 and 6. ×10,000.

Fig. 6.—Dolipore from the tannin region. Note the different staining properties of the septum and the septal swellings. ×15,000.