THE FINE STRUCTURE OF THE MYCORRHIZAS OF *PINUS RADIATA* D. DON

By R. C. FOSTER* and G. C. MARKS†

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Summary

The fine structure of one of two common types of mycorrhiza formed by *Pinus radiata* D. Don is described. The results showed *inter alia* that the ability of a fungus to form mycorrhiza depended on its tolerance to polyphenolic compounds in the so-called tannin layer of the cortex. Hartig net formation is a process in which the invading fungus lysed the middle lamellae and then separated the cells of the host by mechanical action. Evidence of the transfer of carbohydrate from the cortical cells of the host into the fungus in the Hartig net was obtained. Typical dolipore septae were observed both in the mantle and Hartig net hyphae. A new and hitherto undescribed structure was observed in the fungal partner.

I. INTRODUCTION

There is little doubt that ectotrophic and ectendotrophic mycorrhizas are not only beneficial but also necessary in some instances for tree growth (Melin 1953). Typically, a mycorrhizal short root consists of an external hyphal system, a fungal mantle, and a system of intercellular hyphae which lie in intimate association with the host cells. This system of internal hyphae, or "Hartig net", provides a large surface of contact between the fungus and higher plant. Melin and Nilsson (1950, 1952, 1953, 1955) showed that the fungal cells of the mantle functioned like root-hairs and it was possible to follow the passage of nutrients from the external medium into the fungal cells of the mantle and then into the Hartig net. It is in the cortical intercellular spaces of the host occupied by the fungal Hartig net that the food material is exchanged between the two species. Many experiments have revealed both direct and indirect evidence of the passage of inorganic nutrients from the fungus into the root (Harley 1959). Conversely Melin and Nilsson (1957) showed that organic material, possibly carbohydrate, could move from the plant into the fungal sheath. However, nothing is known of the finer structural relationship that underlies this exchange between the host and the fungus.

The objective of this study was to examine the fine structure of the mycorrhizas of *Pinus radiata* D. Don in order to understand better the symbiotic relationship attributed to some mycorrhizas. *P. radiata* was selected for study because it is the major source of softwood in south-eastern Australia.

II. MATERIALS AND METHODS

P. radiata is known to form several types of mycorrhizas (Marks 1965). One of the commonest subtypes, *B*, genus *Ba* (Dominik 1959), found on 1-year-old seedlings

* Division of Forest Products, CSIRO, South Melbourne.
† Forests Commission, Victoria.

was used in this study. The finer lateral roots were washed rapidly in tap water to remove adhering soil, and the mycorrhizal rootlets dissected away. These were cut into small segments about 1 mm long and fixed in the following solutions: (1) 2% $KMnO_4$ in tap water for 2 hr at room temperature; (2) 2% $KMnO_4$ in 0·1M phosphate buffer, pH 6·8, for 2 hr; (3) 2% OsO_4 in phosphate buffer, pH 6·8, for 2 hr at room temperature; or (4) 6·5% glutaraldehyde for 2 hr and post-fixed in 2% OsO_4 for 24 hr at room temperature. This fixed material was washed in phosphate buffer, pH 6·8, dehydrated in t-butyl alcohol, and embedded in Araldite (Glauert and Glauert 1958). The sections were cut on a Siroflex ultramicrotome equipped with a diamond knife and examined in a Siemens Elmiscop I electron microscope at 60 kV. In general, only permanganate fixation preserved the fine structure of both the fungal and host cytoplasm (Wells 1965) and the illustrations are of permanganate-fixed material unless stated otherwise. Some sections were then stained in lead citrate (Reynolds 1963).

III. Results

For the purpose of this investigation the mycorrhiza is divided into four arbitrary regions (Plate 1, Fig. 1): (*a*) the outer mantle where the hyphae are loosely packed and in contact with the soil; (*b*) the inner mantle where the hyphae are closely associated to form a felt-like tissue; (*c*) the so-called "tannin" layer, and (*d*) the Hartig net proper, in which the fungal hyphae are in contact with the living cells of the host.

(*a*) *The Outer Mantle*

In this region the hyphae were distinctly separated from each other (Plate 1, Fig. 2) and the cell walls were frequently thickened (Plate 1, Fig. 3). The outer surface of the wall was incrusted with electron-dense material of unknown nature, possibly soil particles or mucilage produced by the hyphae (Moore 1965). Bacterial cells were associated with the surface of the outer layer of hyphae (Plate 1, Fig. 2). Usually these hyphae were devoid of cytoplasmic contents. In general, the fine cytoplasmic structure was similar to that of other basidiomycetous species described in the literature (Plate 1, Fig. 3). However, the organelles were scattered and sparse (Plate 1, Fig. 3), and had no obvious orientation. The cytoplasm was bounded by a well-defined plasmalemma enclosing a granular ground plasm rather than a double-layered ectoplast as in *Schizophyllum commune* Fries (Wells 1965). Vacuoles, when present, were bounded by a tonoplast which was continuous with the elements of the endoplasmic reticulum so that there was little distinction between the two types of membrane, at least in permanganate-fixed material.

In some cases granular substances were deposited about the periphery of the vacuole (Plate 1, Fig. 3). The fungal nuclei were surrounded by the typical perforated double membrane in which large gaps may occur as in *Schizophyllum commune* (Wells 1965) (Plate 1, Fig. 3). Chromatin bodies could not be distinguished within the fungal nucleus, in contrast to host nuclei, where they were prominent (Plate 9, Fig. 6). The mitochondria were typically rounded in osmium-fixed hyphae. The Golgi (dictyosome) was very uncommon in the fungal associate and no structure which could be unequivocally identified as this organelle was observed in the hyphae of the mantle or within the Hartig net, although they were abundant within the host cells. Lomasomes

were uncommon in the hyphae and elements of the endoplasmic reticulum were not very abundant. The occurrence of two types of deeply stained material was of particular interest. One was very electron dense and it was surrounded by a single membrane, recalling the "oil globules" of Wells (1964). The other was less electron dense and it did not have an enclosing membrane. This latter material was found to be similar to glycogen granules of fungal cells (Wells 1964; Foerster, Behrens, and Airth 1965) and in animal tissue (Drochmans 1960; Kairer and Cox 1960). The other material has been tentatively identified as oil deposits. Towards the inner regions of the outer mantle where the hyphae became more compacted to form the felt-like inner mantle, the hyphae were embedded in an amorphous matrix (Plate 4, Fig. 2). This proved to be a technical hindrance since it stopped the penetration of both fixative and embedding medium, and this caused sections to sometimes disintegrate at this point. The nature of this matrix has not been determined but it is thought to be composed of either a fungal gel or mucilage (cf. Moore 1965). Hyphae within the matrix were widely spaced, had moderately thick walls, and were in general very poorly preserved by all the fixatives used. In some mycorrhizas the matrix was rather indistinct and limited in extent.

(*b*) *The Inner Mantle*

In the inner mantle the cells tended to be closely packed so that in many parts there were no spaces between the hyphae (Plate 2, Fig. 2). This layer varied in thickness and in some associations it was only one or two hyphal layers thick. In general, there was a tendency, more pronounced in some mycorrhizas than others, for the hyphae to be transversely orientated with respect to the axis of the rootlet (Plate 2, Fig. 1). The walls, although slightly thinner than those of the hyphae of the outer mantle, consisted of two layers (Plate 2, Fig. 2). The hyphal septa occasionally showed dolipores (Moore and McAlear 1962; Bracker and Butler 1963) (Plate 2, Fig. 1; Plate 3, Fig. 3; Plate 4, Fig. 1). In the dolipore the sub-cap matrix was electron-lucent and connections with the endoplasmic reticulum were well developed. The septa in sections not including the dolipore had at least four layers (Plate 3, Fig. 4). They showed a deeply stained ectoplast layer, a septal plate, and central transparent lamella, and between the ectoplast and the septal plate there was an electron-lucent layer. The whole structure therefore resembles that recorded for *Rhizoctonia solani* Kühn (Bracker and Butler 1963). In some areas the hyphae were separated by thin layers of deeply stained materials that were different to the mucilagenous matrix of the outer mantle (Plate 2, Fig. 2). Such cells were restricted to isolated pockets. In some instances where the intercellular spaces were large these materials were in the form of needle-shaped crystals and it is believed that they may be polyphenolic substances.

The cytoplasmic structure of the hyphae in this region was similar to that in the outer mantle, but the organelles were more numerous and vacuoles were less translucent. The distribution of the storage products was of particular interest. They were of two types. The first appeared as thick-walled, hollow, deeply stained vesicles (Plate 2, Fig. 1, arrowed) and were abundant in the mantle hyphae but occurred rarely in the Hartig net. They were similar to the "unidentified cytoplasmic inclusion bodies" found in the root cap cells of maize by Whaley, Mollenhauer, and

Leech (1960) and in the subepidermal cells of *Avena* coleoptile by Wardrop and Foster (1964). These have been identified as lipid bodies by Frey Wyssling, Greshaber, and Muhlethaler (1963) but are very unlike the oil bodies of Wells (1964) which were electron translucent, having a thin membrane, or Wells (1965) which are uniformly electron dense.

Glycogen was the second storage product and its distribution was most significant. The glycogen deposits were mainly confined to the peripheral cytoplasm as were the oil bodies of Wells (1964). There were, however, isolated pockets in the hyphae (usually lateral branches) densely packed with the material. Although glycogen was seen infrequently in the outer mantle it was densely and evenly distributed in the hyphal cells of the inner mantle and Hartig network.

Nuclei where present were large, tending to fill the cross-sectional area of the hyphae, and they were distinguished by their smooth outline. There were indications that the nuclei were able to move through septa (Plate 3, Fig. 2) having simple pores as described by Giesy and Day (1965). In Plate 3, Figure 1, either the nucleus has divided recently or there is a close nuclear association in the dikayrotic hypha. In some cells the vacuoles were irregular in outline, and the tonoplast tended to merge with parallel membranes which are indistinguishable from those of the endoplasmic reticulum.

(*c*) *The "Tannin" Cell Zone*

Most descriptions of mycorrhizas frequently refer to deeply stained deposits in the outermost layer of cortical cells. This layer has been referred to as a "tannin layer". In subtype *B*, genus *Ba*, it usually consisted of a single layer of cells. However, in some specimens it was more extensive and both the fungal and host cells showed considerable abnormalities in this region. It was noticed that the host cells in this zone were large and filled with deeply stained materials which are believed (Foster and Hillis, unpublished data) to be polyphenolic deposits (Plate 1, Fig. 1; Plate 4, Fig. 1). The deposits may be partially crystalline and their hardness and inpenetrability present technical difficulties in embedding and sectioning. The outer host cells of the tannin layer were devoid of organelles and their developmental stages show several unusual features to be described elsewhere.

In some regions there was a very sharp transition in hyphal structure between the mantle and "tannin" region (Plate 4, Fig. 1). These modifications were seen not only in gross morphology but also in the cytoplasmic fine structure (Plate 5, Figs. 1 and 2).

The morphological changes will be described later, attention in the present paper being confined to the cytoplasmic structure. The majority of the hyphae were filled with deeply stained amorphous materials and their organelles appeared to degenerate so that they could be distinguished within the cells only with difficulty (Plate 4, Fig. 1; Plate 5, Fig. 1). Some of these hyphae appear crushed and distorted and in some the organelles have completely disappeared, the whole lumen being filled with electron-dense material (Plate 4, Fig. 1). These hyphae are thought to be either dead or moribund. Between these moribund hyphae there were individual cells devoid of the dark amorphous material, and their cytoplasm was similar in structure to that found in the outer mantle (Plate 5, Fig. 1). There were some differences,

however, particularly in the abundance of the organelles. The endoplasmic reticulum was frequently more common and the nuclei were more prominent. The cells contained abundant glycogen even when they were filled with the dark material (Plate 4, Fig. 3). Considerable interest, however, centred around unusual vesicle-like structures. They had a single membrane, but unlike normal vacuoles they had a dense deposit within, and contained tubular cristae-like profiles (Plate 5, Figs. 1 and 2, arrows). These vesicles were larger than mitochondria and had an irregular outline. Normal mitochondria occurred alongside these vesicles so it seems unlikely that they are a fixation artefact. As far as we can ascertain such structures have not been described previously and their nature and function remains uncertain. In their electron density and possession of a single membrane they resemble the "unidentified cytoplasmic inclusion" of Wells (1964).

It was observed that in this region the dolipore could exercise control over the movement from cell to cell of the deeply staining materials. In Plate 4, Figure 3, two dolipores separate three cells. The upper two cells were devoid of the stained material whilst the lower was impregnated. It would seem that even though a septal pore and discontinuous parenthosome exist the dolipore is either impervious to materials or can exert a selective action on the passage of materials through it.

It appeared that the fungal hyphae were adversely affected by the polyphenols secreted by the cortical cells in this region since very few hyphae had the normal appearance. However, once the hyphae penetrated the "tannin" layer and entered the intercellular spaces of the inner cortex their usual morphological and cytoplasmic structure reappeared. It is presumed that the reduction in polyphenolic content of the cells in the inner cortical layers accounts for the re-appearance of the normal hyphal structures.

(*d*) *The Hartig Net*

In the Hartig net region, the cells of the host and fungus come into intimate contact (Plates 6, 7, and 8), and it is assumed that it is in this area that the transfer of materials between the two organisms takes place. In the association examined it was observed that the fungal hyphae were wholly confined to the intercellular spaces and in none of the roots were haustoria produced. In the parasitic associations of Ehrlich and Ehrlich (1963) and Peyton and Bowen (1963), there was a marked reaction of the host tissues to the presence of the fungus in that material which was secreted by the host cytoplasm to form a deeply stained "zone of apposition" around the invading fungal hyphae. No such apposition zone was observed in the mycorrhiza but the host cells in juxtaposition to the mantle underwent some change in their fine structure. In some of these inner cortical cells, the cytoplasmic organelles were disorganized (Plate 7, Fig. 1) and abundant tannin-like materials were produced (Plate 9, Fig. 1). These effects were not fixation artefacts because in the same region perfectly fixed cells lay adjacent to those showing disorganized organelles and even within the same cell, parts of the protoplast showed normal cytoplasmic structures (Plate 9, Fig. 4), while in other parts of the same cell they were disorganized (Plate 9, Fig. 1). Elsewhere in the host cells of the Hartig net zone it was observed that there was a very large vacuole in each cell and often it was lined with polyphenolic materials

which were sometimes aggregated into large dense masses (Plate 6). The cytoplasm was confined to a thin peripheral layer. It contained abundant endoplasmic reticulum, mitochondria, and Golgi apparatus. Frequently the nuclei were very large (Plate 9, Fig. 6), rounded, without the usual invaginations and the chromatin bodies were very strongly stained. Both the tonoplast and the plasmalemma were intact and the cells had the same appearance as those in a similar position in uninfected roots (Foster, unpublished data). The tonoplast surrounding the vacuole was usually convoluted so that occasionally the plasmalemma and tonoplast come into contact (Plate 8, Figs. 2 and 3). In this region the host cells contained amyloplasts which were always devoid of starch (Plate 7, Fig. 1; Plate 8, Fig. 2). This was quite unlike those in uninfected roots (Plate 8, Fig. 1). The mitochondria of the host were often elongated and richly endowed with cristae, suggesting active metabolism.

The most obvious alteration in the host cells occurred in the cell walls where the walls of contiguous cells separated along the middle lamella. Apparently the walls were separated mechanically in advance of the penetrating hyphae (Plate 6; Plate 8, Fig. 3) following lysis of the middle lamella. No evidence of wall decomposition was observed in surface replicas of the host cells (Foster and Marks, unpublished data). Where the hyphae penetrated between host cells they often appeared wedge-shaped (Plate 8, Fig. 3) and it was frequently observed that the hyphal cells in recently penetrated tissue were very small and closely packed, having an angular outline (Plate 7, Fig. 2) whereas those in the larger intercellular spaces had a greater diameter and a more rounded outline with spaces between the individual hyphae. As intercellular penetration continued, the older hyphae became somewhat rounded and the contiguous host cells were separated from one another (Plate 8, Fig. 4).

The fine structure of the fungal hyphae within the Hartig net differed in detail from that in the more peripheral regions of the association. In newly penetrated regions the small angular cells were packed with glycogen so that other organelles were somewhat sparsely scattered (Plate 7, Fig. 2). The vacuoles, when present, were small and remarkably electron transparent and free from granular deposits when compared to the vacuoles of the host (Plate 6). There was some indication that the fungal mitochondria divided actively and in the older parts of the Hartig net they were more elongated and very rich in cristae. They were not so large and abundant as in definitely parasitic associations (Ehrlich and Ehrlich 1963). The endoplasmic reticulum was as abundant as in the more recently penetrated areas. It mainly consisted of parallel lamellae but these frequently opened out into vacuoles with electron-transparent contents and we believe that there is very close connection between the vacuoles and the endoplasmic reticulum in this tissue. Occasionally, the deeply stained "lipid bodies" are present within the cells (Plate 6; Plate 9, Fig. 3). For the most part, however, the storage reserve appeared to be glycogen and a significant difference between the cells of the Hartig net and those of the mantle is that the former was always packed with glycogen, so that frequently little ground plasm could be seen. In permanganate-fixed material the edge of the glycogen deposits was granular and this effect was very marked in glutaraldehyde-fixed specimens (Plate 9, Fig. 5).

Within the host, dolipore septa were found and they were similar to those in the peripheral mantle (Plate 9, Figs. 2 and 3). As far as we are aware this is the first

time they have been seen in a basidiomycete hyphae lying within host tissues. Hyphae comprising the Hartig net, especially those in the larger intercellular spaces, frequently had nuclei visible (Plate 6).

IV. Discussion

Fine structure studies have been made on the host–parasite relationship of several species of obligate parasites of higher plants (Ehrlich and Ehrlich 1963; Peyton and Bowen 1963; Berlin and Bowen 1964) but there seems to be no work published on the symbiotic ectotrophic mycorrhizas. In the present investigation one or two of the commoner types of mycorrhizal associations (Bowen 1963; Marks 1965) of *P. radiata* is considered. Apart from the absence of haustoria there are three prominent aspects of fine structure in which this mycorrhiza differs from the obligate parasites as described by Berlin and Bowen (1964) and Peyton and Bowen (1963). In *Albugo* (Berlin and Bowen 1964) the hyphae were filled with abundant large mitochondria, whereas in the structures seen by us the mitochondria were small when compared with the cross-sectional area of the hyphae. Furthermore, in the material under consideration, especially in the hyphae of the Hartig net, large amounts of glycogen accumulated. There was no evidence of disorganization or disruption in the cytoplasm of the host cells and the mycorrhizal fungus was readily tolerated by the host. There was no apposition of materials by the host cytoplasm tending to isolate the fungal hyphae as in the parasitic associations.

A difficulty exists in this type of investigation with naturally formed mycorrhizas. There is always the uncertainty that the fungus in the mantle may not be the same as that forming the Hartig net. Many types of association are formed on the roots of *P. radiata* (Marks 1965) and only in very rare instances have situations been observed where two or more distinct types of ectotrophic mycorrhizas form on the same root. Thus, it appears that once a root is infected with a particular type of mycorrhiza-forming fungus a stable entity results. Studies of the anatomy and fine structure of other mycorrhizas (of *P. radiata*) showed that mantle characteristics are always associated with particular Hartig net characteristics (Foster and Marks, unpublished data). Thus it is believed unlikely that the fungus forming the mantle could be different to that forming the Hartig net.

There has been much conjecture as to why forest trees form mycorrhizal associations (Kelley 1950). The most widely accepted theory explaining the existence of this association originated from the work of Björkman (1940, 1942, 1944). He assumed that the fungus obtained its carbohydrate from the tree rootlet and it was the sugar content of the root that determined whether a mycorrhizal association would form. According to him, an excess of soluble sugars in the root stimulated the fungus to penetrate into the root and establish the association; conversely, a deficiency of soluble sugars would suppress the development of the mycorrhizal association. A weakness in this concept is that this stimulus lacks specificity and that the fungus has "advance knowledge" of the existence of the carbohydrate (Harley 1959). However, despite this weakness, the basic assumption received support from Melin and Nilsson (1957) who showed that $^{14}CO_2$ supplied to photosynthesizing leaves caused radioactivity to appear in the fungus mantles after 5 hr.

Björkman's concepts about the movement of carbohydrates were different from those of MacDougal and Dufrenoy (1944) who maintained that the mycorrhizal fungi were able to synthesize carbohydrate which passed from the litter into the mantle and thence into the body of the root. Handley and Sanders (1962) re-examined Björkman's hypothesis. They found little change in the reducing sugar content in the roots inoculated artificially and grown aseptically in light of varied intensity. They suggested yet another hypothesis, namely, that the accumulation of these reducing substances following mycorrhizal formation was the sequel to infection rather than the cause of it. This situation could be analogous to infection of the foliar organs with obligate parasites (Allen 1960). Melin (1959) concluded after prolonged and exhaustive study that the formation of ectotrophic mycorrhizas depended not only on an excess of soluble carbohydrates in the root but also on root metabolites that both promoted and inhibited the growth of the fungal component.

The results reported here give visual evidence of carbohydrate exchange. Areas of the cortex can be seen free of fungal involvement, and the cells contain amyloplasts filled with starch (Plate 8, Fig. 1). Conversely, those cortical cells in juxtaposition to fungal hyphae have empty amyloplasts (Plate 7; Plate 8, Fig. 2) while the fungal cells are packed with glycogen. In contrast to those fungal cells in the Hartig net, the cells of the mantle contain lesser amounts of this material and there appears to be a diminution in glycogen content in passing from the inner fungal cells of the Hartig net to the outside. Thus it appears that the fungus withdraws starch-like food material from its host and that the host could be the major carbohydrate source for the fungus. This observation supports the Swedish work. The photographs obtained from mycorrhiza subtype *Ba* shows that glycogen accumulates in the fungus without physical disruption of the host wall or the organelles enclosed within the host. It is certain that the carbohydrate is reduced to a soluble form in order to effect this transfer. Thus, Handley's results obtained from analysis of reducing content would depend on the age at which infection occurred and metabolic activity of the fungus and the root, and on the ability of the latter to keep the fungus supplied with carbohydrate and the ability of the fungus to extract and metabolize it.

Most mycorrhizal fungi are incapable of synthesizing cellulases excepting under special circumstances. Thus, they do not as a rule use cellulose as an energy source. However, they do secrete pectolytic enzymes *in vitro* and this may be considered as a natural occurrence. Unfortunately, there is no evidence as to how these enzymes operate during intracellular penetration and Hartig net formation. Plate 8, Figure 3, suggests that the fungus loosens the cementing substance between contiguous cortical cells. Subsequently, the fungus appears to wedge itself into the minute fissures so formed and then force the cells apart, splitting the intercellular substance (Plate 8, Fig. 4). Considerable distortions occur in the fungal cells during this process. Thus, it appears that penetration in the *Ba* type is primarily a mechanical process following pectolysis of the middle lamella.

This mechanical process would be greatly facilitated if the growth and expansion rate of the cortical cells were reduced. Thus, if growth and expansion of the root were slowed down prior to intracellular penetration by the secretions of surface fungi, Hartig net formation may occur. Such a change in the growth potential could be

brought about by auxins secreted by the mycorrhizal fungus while still on the root surface (Slankis 1959).

This mechanical explanation for Hartig net formation may account for the labyrinthiform appearance of the Hartig net in surface view and explain the reason why actively growing long root tips do not form mycorrhizas, but the associations do form in the long root when growth slows (Robertson 1954). This concept will conform to Wilson's hypothesis (Harley 1959) that ectendotrophic mycorrhizas form as a sequel to a change in the growth potential of the long root.

V. References

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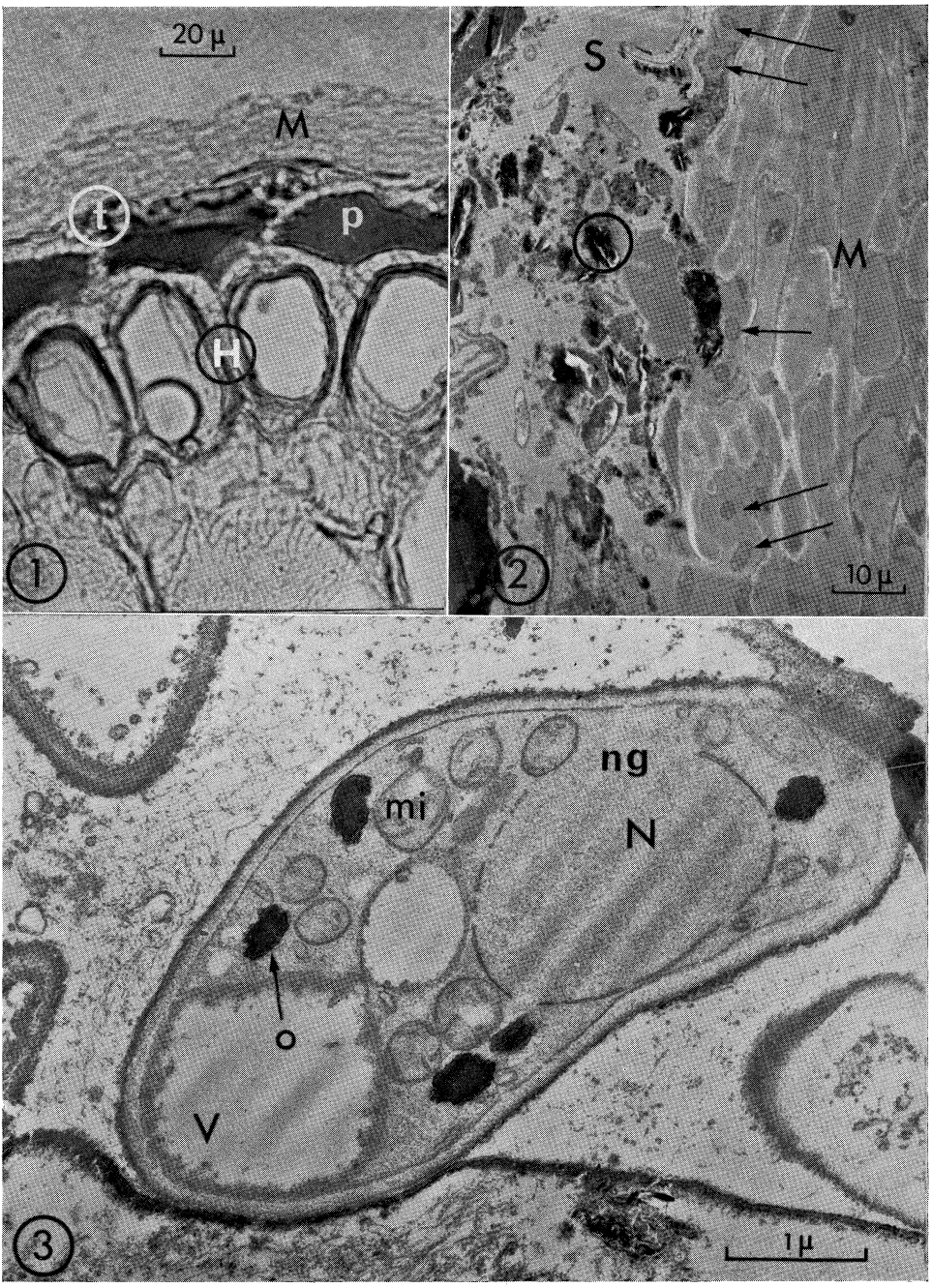
EXPLANATION OF PLATES 1–9

All figures except Plate 1, Figure 1, are electron micrographs and all are of permanganate-fixed material without lead citrate stain unless otherwise stated. The explanation of lettering used for the labelling of the various structures is as follows:

c	dolipore cap	*N*	nucleus
cl	clear layer of septal wall	*Ng*	nuclear gap
d	dolipore structure	*o*	oil body
e	ectoplast	*p*	polyphenolic substances
er	endoplasmic reticulum	*pa*	plasmalemma
fp	fungal cells filled with polyphenols	*pl*	plastid
fw	fungal cell wall	*S*	soil
g	glycogen	*sm*	sub-cap matrix
H	Hartig net region	*sp*	septal pore
Ht	host tannin cell	*spl*	septal plate
HW	host cell wall	*ss*	septal swelling
Hy	hypha	*St*	starch
M	mantle region	*t*	tannin layer
mi	mitochondrion	*U*	unstained layer of septum
ma	matrix "mucelagenous" region	*V*	vacuole
		vt	thick-walled vesicle

FINE STRUCTURE OF *P. RADIATA* MYCORRHIZAS



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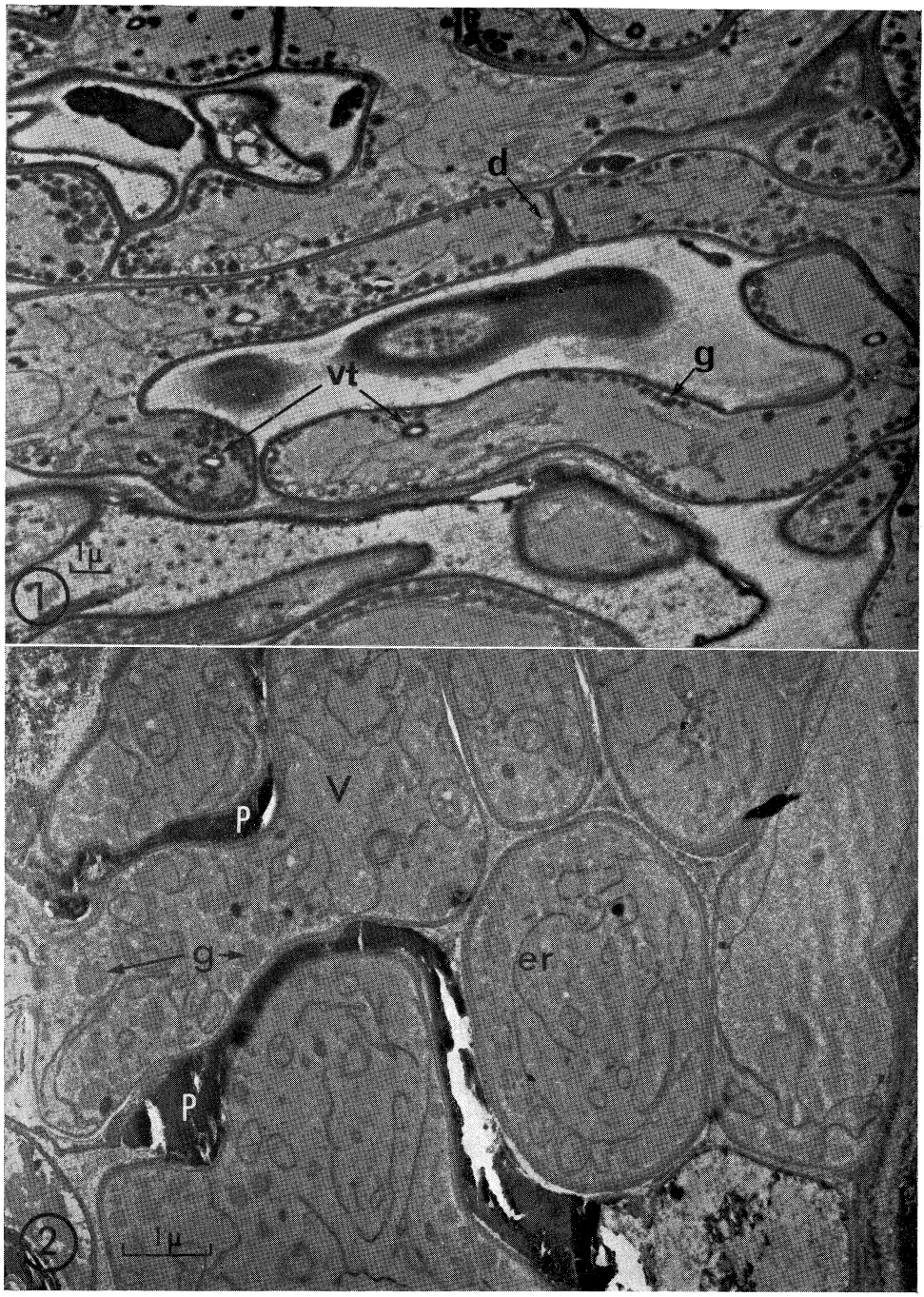

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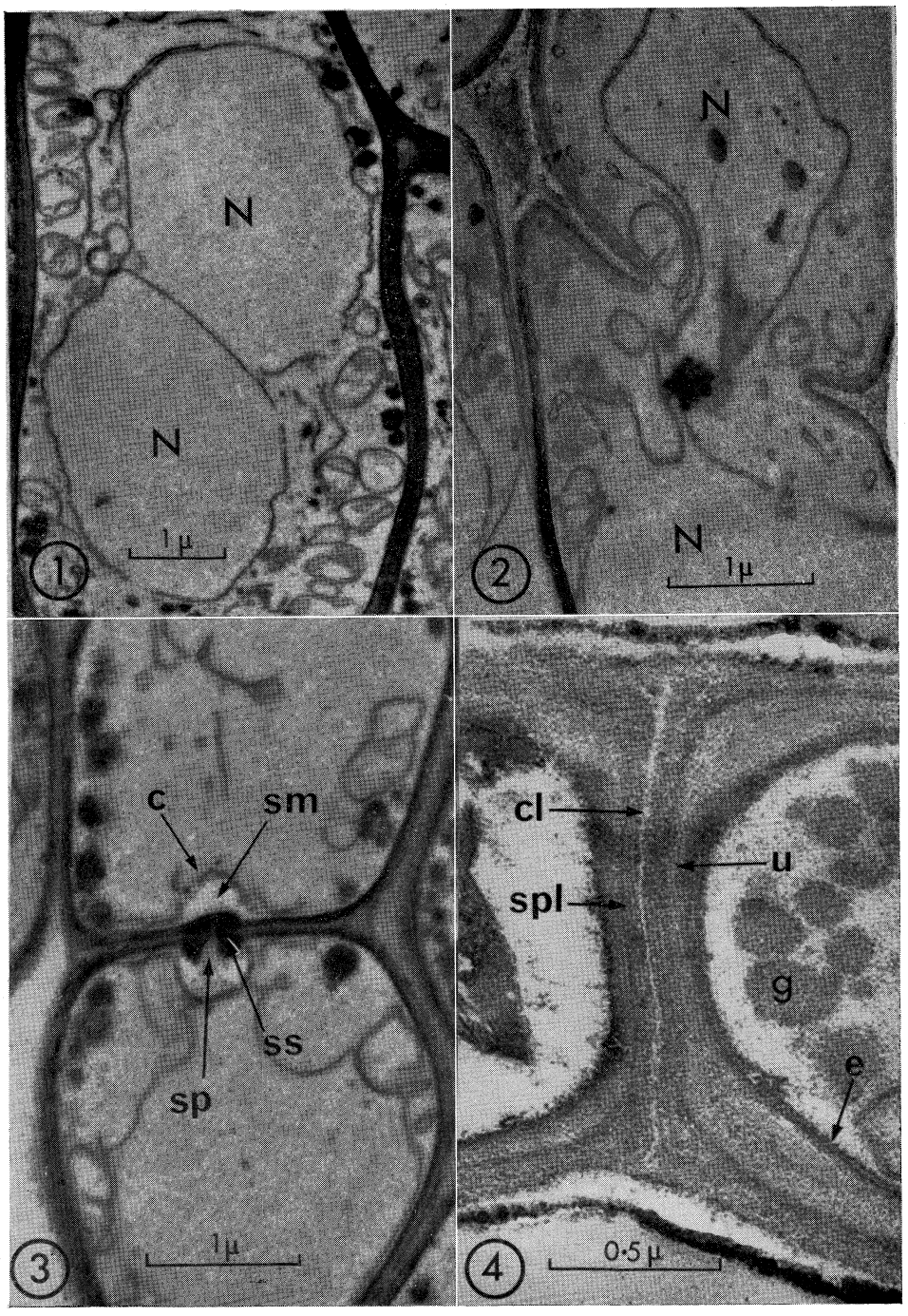

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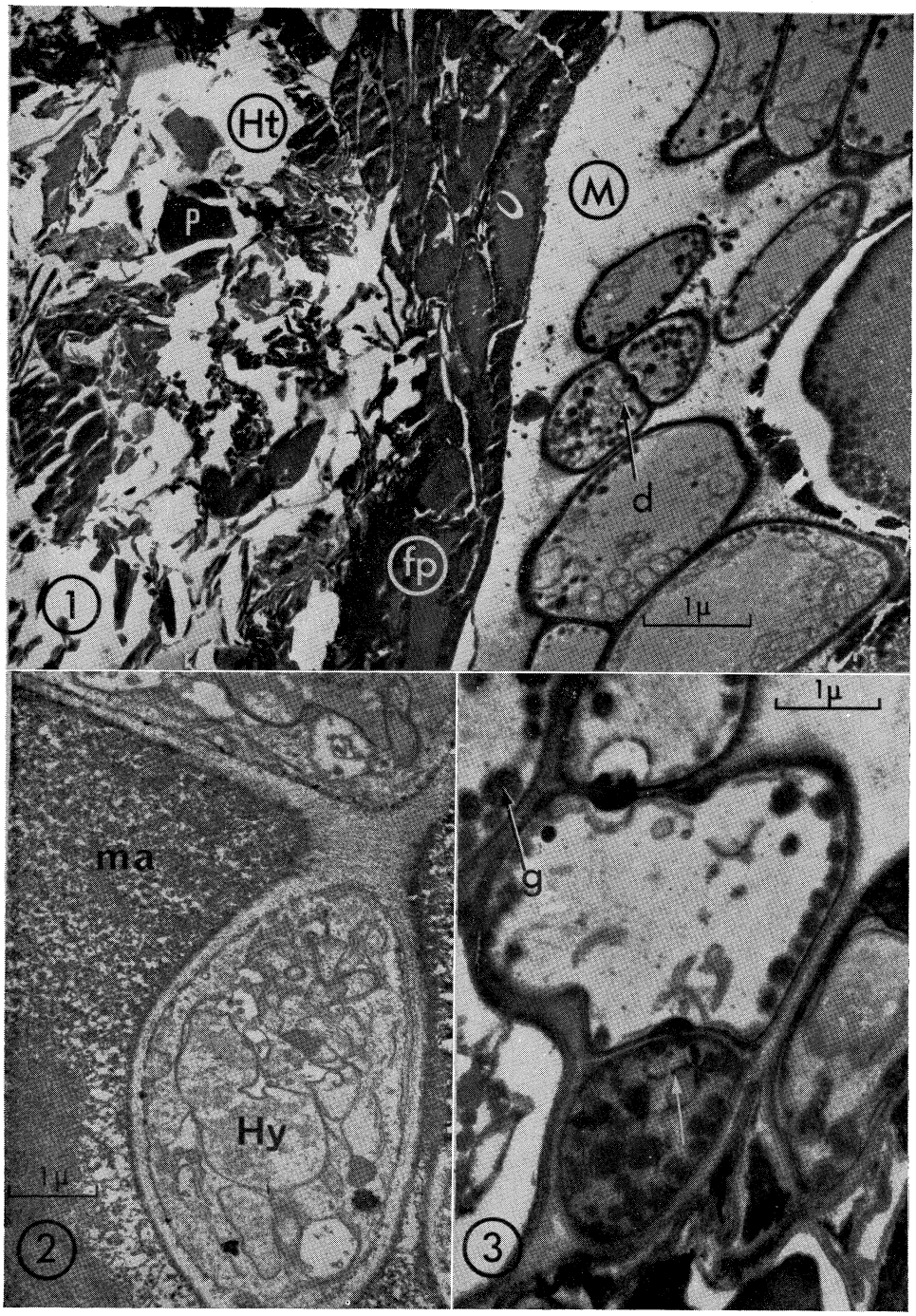

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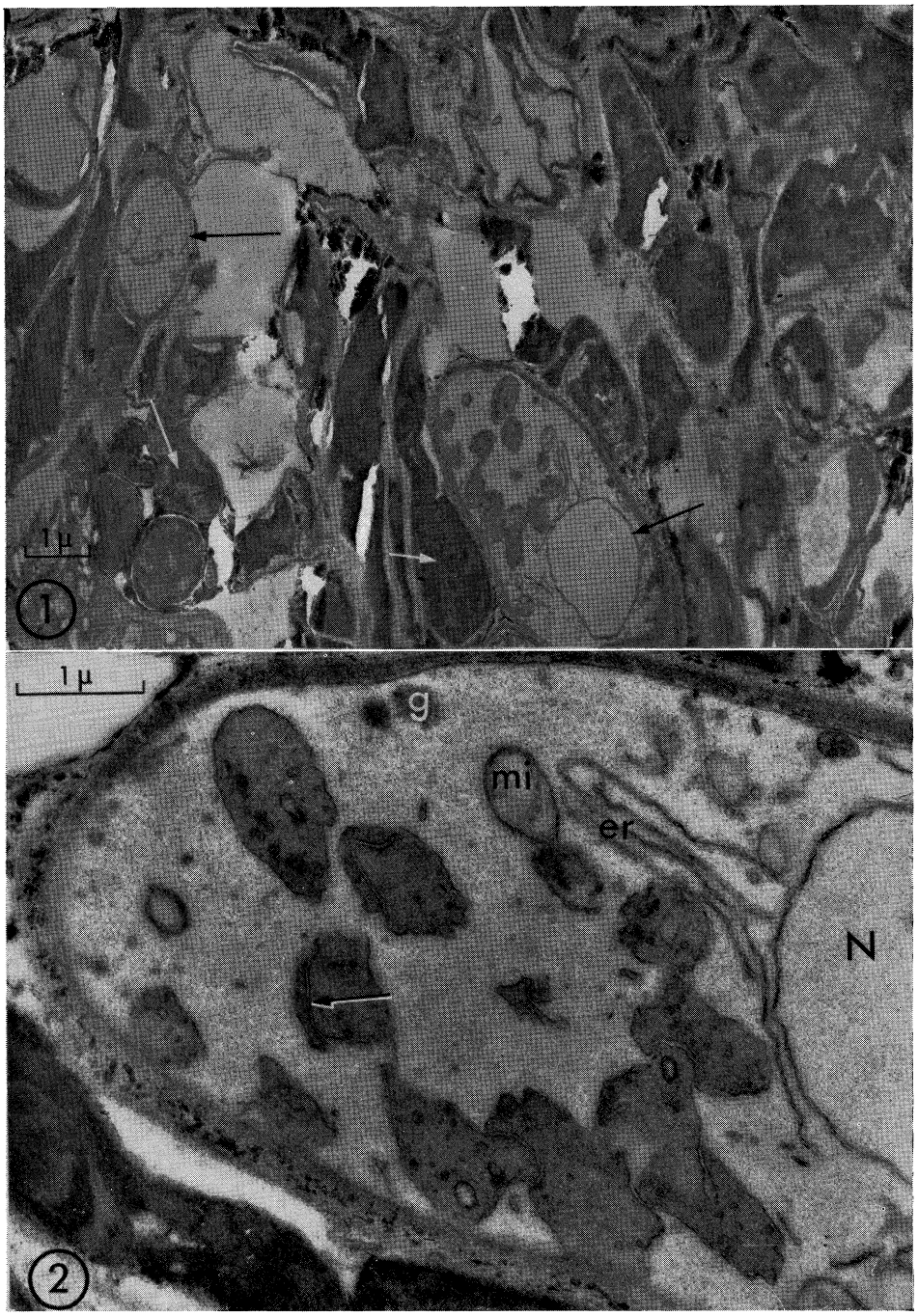

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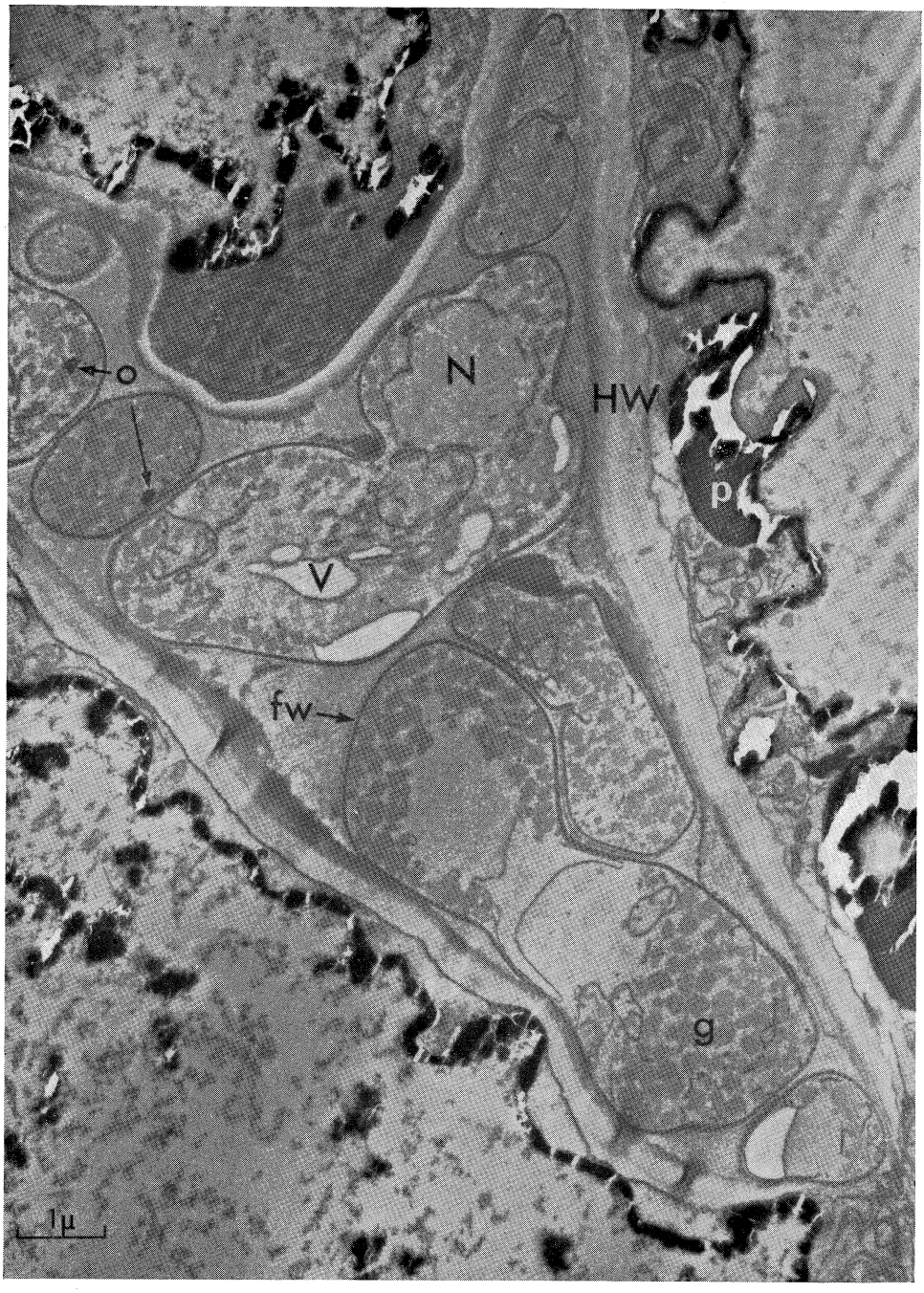

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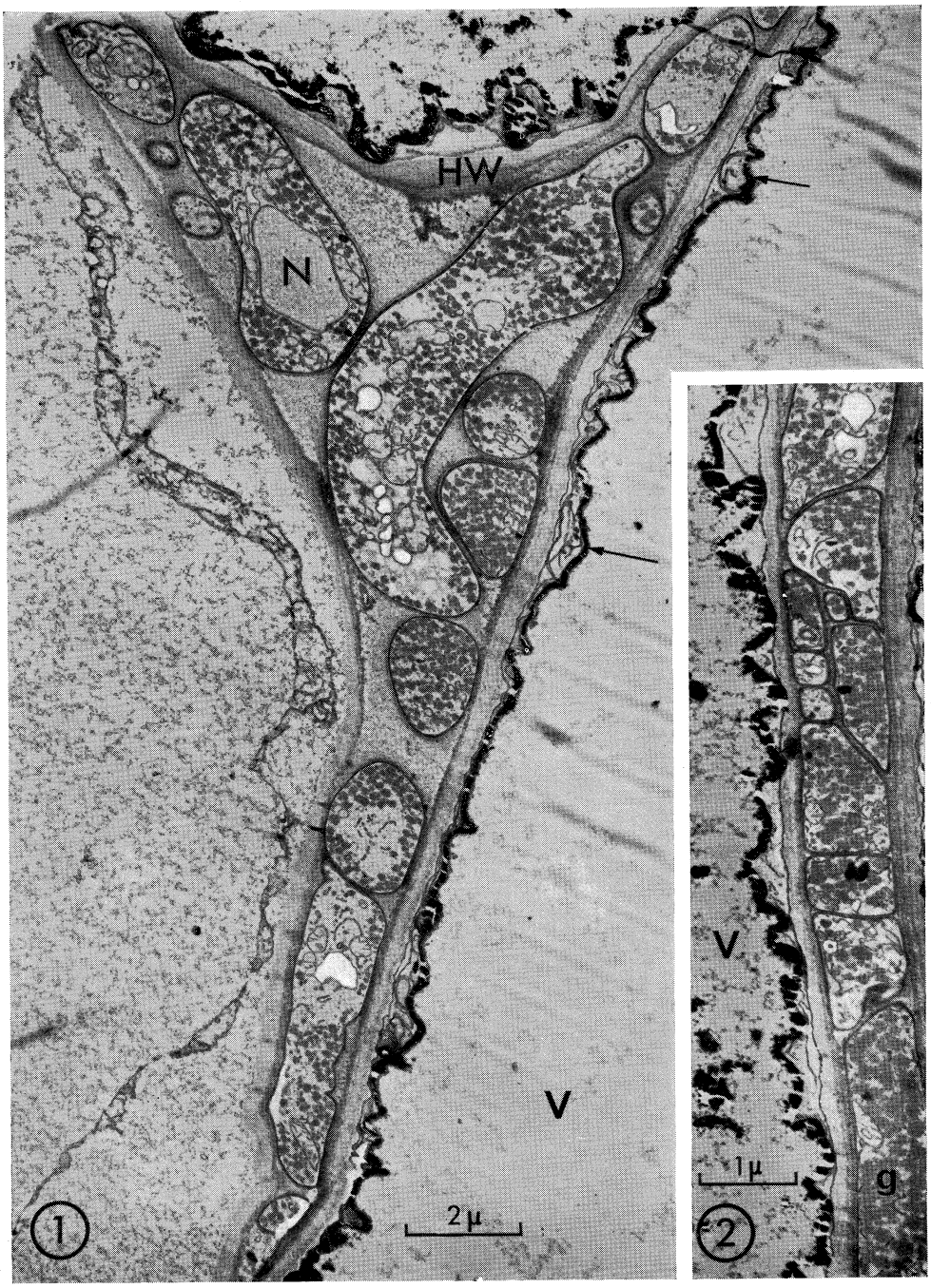

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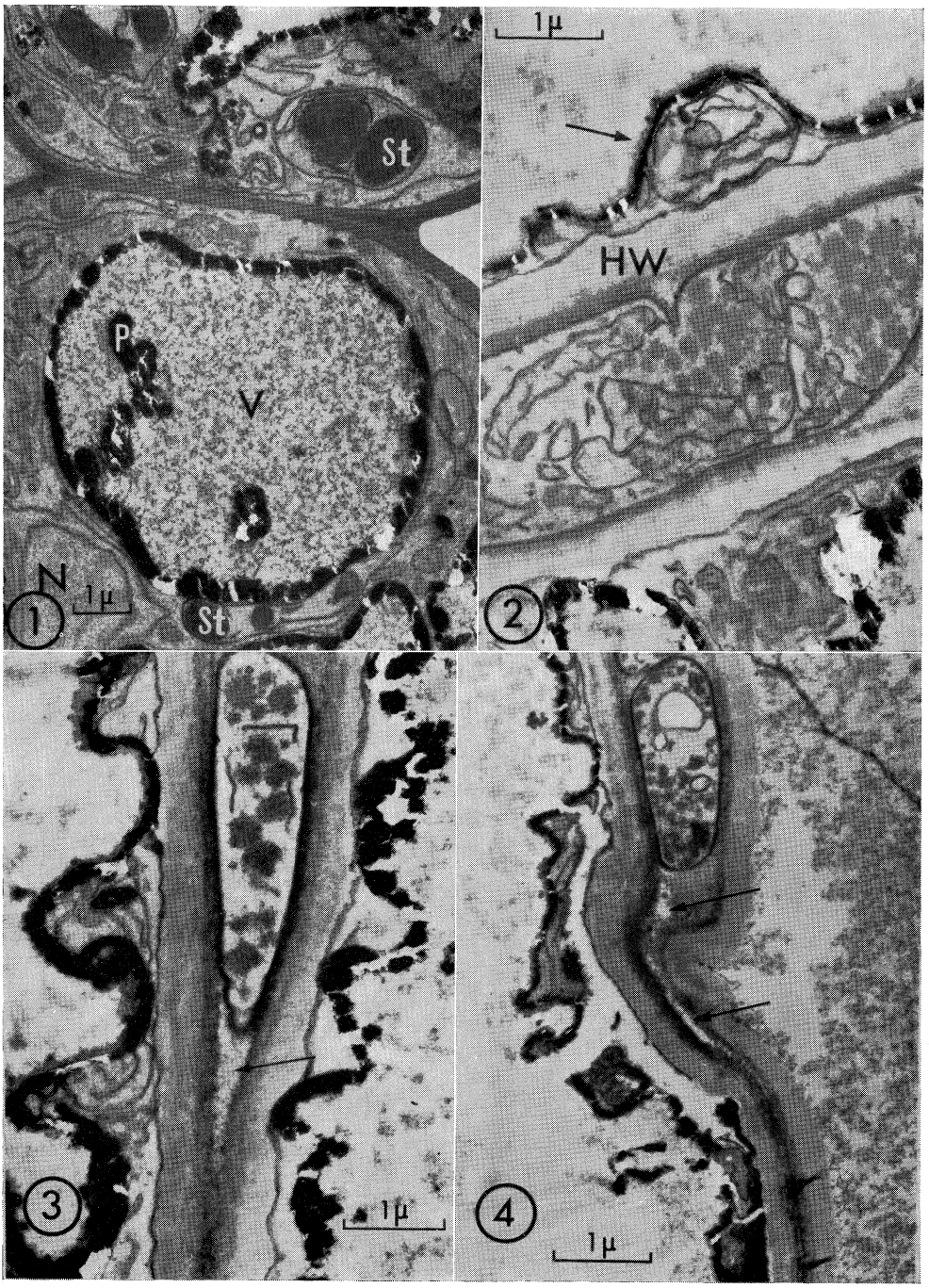

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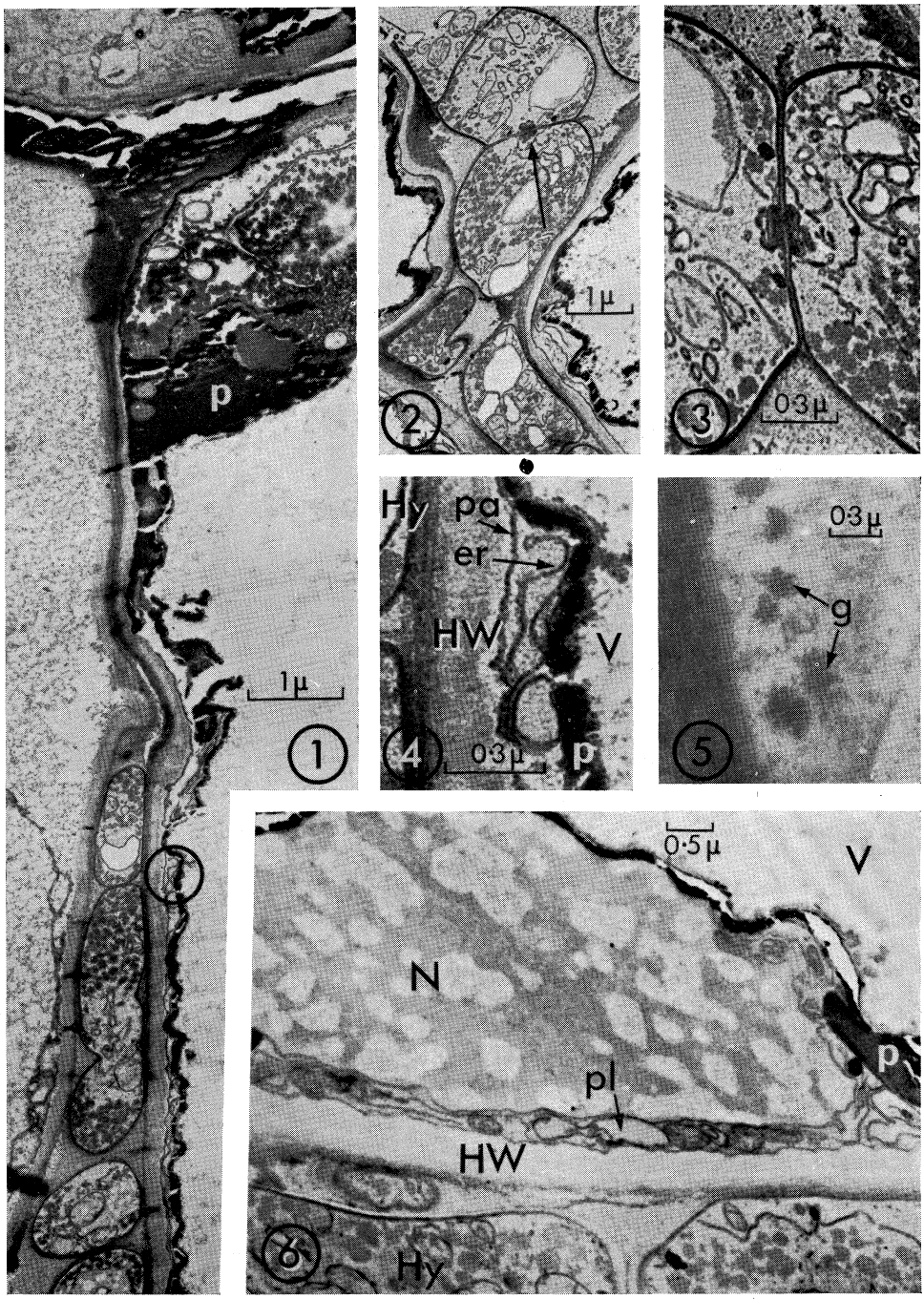

PLATE 1

Fig. 1.—Transverse section through the mycorrhizal association of subtype *B*, genus *Ba*. Ringed: tannin-filled hyphae. Light photomicrograph. × 500.

Fig. 2.—Transverse section as in Figure 1, but fixed in osmium tetroxide. Arrows indicate associated bacteria. Ringed: clay mineral particle. × 1000.

Fig. 3.—Hypha of outer mantle. × 19,000.

PLATE 2

Details of mantle region

Fig. 1.—Outer compact mantle region. Lead stained. × 6000.

Fig. 2.—Inner mantle region. Note polyphenolic material between the hyphae. × 12,000.

PLATE 3

Details of mantle region

Fig. 1.—Closely associated nuclei. Lead stained. × 12,000.

Fig. 2.—Nucleus passing through simple septal pore. × 19,000.

Fig. 3.—Dolipore septum. Lead stained. × 20,000.

Fig. 4.—Details of septal structure. × 50,000.

PLATE 4

Fig. 1.—Transition region between mantle and tannin layer. On the right are cells of the mantle, on the left a large host cortex cell (*Ht*) filled with polyphenolic materials. Between them are fungal cells filled with darkly stained material. Arrow indicates dolipore in mantle hyphae. Lead stained. × 5500.

Fig. 2.—Hyphae embedded in granular "mucelagenous" material. Fixation characteristically poor. × 14,000.

Fig. 3.—Fungal hyphae of "tannin zone" showing three cells separated by two dolipore septa (the lower one indicated by the white arrow). The lower cell is filled with deeply staining material. Lead stain. × 14,000.

PLATE 5

Details of the tannin layer

Fig. 1.—Fungal hyphae of the tannin region showing living (black arrow) and moribund (white arrow) hyphae. Lead stained. × 8000.

Fig. 2.—Detail of Figure 1 showing unusual organelles containing cristae-like tubules (arrowed). Lead stained. × 18,000.

PLATE 6

Hartig net region showing fungal hyphae in the intercellular space between three host cells. Note abundant glycogen in hyphae and polyphenolic masses in host cells. × 12,000.

PLATE 7

Hartig net region

Fig. 1.—Hartig net region as in Plate 6, left-hand side showing dead host cell. Note plastids devoid of starch in host cells (arrowed). Lead stained. × 8000.

Fig. 2.—As in Figure 1, showing angular glycogen-filled fungal cells in a recently penetrated region. Lead stained. × 15,000.

Plate 8

Fig. 1.—Parenchyma from non-mycorrhizal region of *Pinus radiata* root showing plastids with abundant starch. Lead stained. × 7000.

Fig. 2.—Similar cell from Hartig net showing a plastid almost devoid of starch (arrowed). × 14,000.

Fig. 3.—Wedge-shaped hypha penetrating the middle lamella between two living host cells. Arrow indicates apparent lysis of middle lamella. Lead stained. × 15,000.

Fig. 4.—As in Figure 3, later stage. Note rounded hyphae and splitting of middle lamella region (arrowed). Lead stained. × 15,000.

Plate 9

Details of the Hartig net region

Fig. 1.—Penetration of fungal hyphae between host cells. Cell on the left dead; cell on the right alive in lower portion but secreting polyphenols in upper region (see also Fig. 4). Lead stained. × 12,000.

Fig. 2.—Hartig net region showing dolipore between two fungal cells. Lead stained. × 10,000.

Fig. 3.—Details of Figure 2. × 30,000.

Fig. 4.—Detail of ringed area of Figure 1 showing normal organelles in lower part of cell. × 30,000.

Fig. 5.—Fungal hypha fixed in 6·5% glutaraldehyde and post-fixed in 2% osmium tetroxide, showing granular glycogen deposit. × 30,000.

Fig. 6.—Detail of host cell showing nucleus with prominent chromatin bodies and plastid (*pl*) devoid of starch. × 10,000.