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Mycorrhiza

Phosphorus relations of roots and mycorrhizas of *Rhododendron maximum* L. in the southern Appalachians, North Carolina

John Dighton[1] **and David C. Coleman**[2]

[1] Institute of Terrestrial Ecology, Merlewood Research Station, Grange-over-Sands, Cumbria LA11 6JU, UK
[2] Department of Entomology, University of Georgia, Athens, GA 30602, USA

Summary. The mycorrhizal associations of *Rhododendron maximum* in the southern Appalachian mountains were studied in relation to the supply and demand of phosphate at three altitudes. A variety of mycorrhizal associations are described together with the ability of the differing mycorrhizal types to produce phosphatase enzyme, which was inversely related to the availability of inorganic phosphate determined by a root bioassay, as Ectomycorrhizal associations were shown to have a higher phosphatase production potential than other mycorrhizas. The availability of inorganic phosphate at different altitudes is related to turnover of organic matter and fixation capacity of the mineral soil. It is speculated that the ability of *R. maximum* to associate with a range of mycorrhizal associates is likely to improve species' fitness and enhance its competitive ability.

Key words: *Rhododendron maximum* – Phosphatase – Root bioassay – Altitude – Southern Appalachians

Introduction

Rhododendron maximum L. is an invasive species occupying gaps created in the cove forests on the slopes of the southern Appalachian mountains. Once *Rhododendron* has become established it forms a dense canopy which inhibits regrowth of the native tree species, leading to large monospecific stands of *R. maximum*. It appears to thrive on the acidic and nutrient-poor soils of this region and indeed may intensify acidification and leaching of cations in its understorey (Boettcher and Kalisz 1990). Little, however, is known of its mycorrhizal associations or its abilities to compete with the native forest flora for nutrients (Brundrett 1991). *Rhododendron* leaves are long-lived and its litter is slow to decompose and, therefore, is a source of immobilization of essential nutrient elements (Monk et al. 1985). Close associations between decomposition processes and the roots of *Rhododendron* could optimize uptake of mineral nutrients and prevent leaching from the rooting zone. Mycorrhizal associations of roots are involved in organic matter decomposition (Bajwa and Read 1986; Dighton et al. 1987; Read 1991) and the acquisition of nutrients from organic matter (Dighton 1991). Such a close mutualistic association could improve the fitness (Allen 1991) of *Rhododendron* in nutrient-poor acidic soils, where the availability of soluble inorganic nutrients is likely to be low.

It has been shown that *Rhododendron* roots have the ability to be associated with a range of mycorrhizal partners (Johnson et al. 1980; Largent et al. 1980). The nature of the mycorrhizal association of *Rhododendron* roots may influence the ability of the roots to access nutrients from either inorganic (Dighton and Harrison 1990) or organic resources by virtue of enzymatic capabilities of the fungal partner (Linkins and Antibus 1981; Giltrap 1982; Dighton 1983; Bajwa et al. 1985; Abuzinadah and Read 1986; Dighton et al. 1987).

Phosphorus may be a limiting factor for plant growth in many upland acidic soils (Harrison 1987). Its availability depends on the interaction between the rate of mineralization from organic matter and mineral forms and the rate of fixation onto soil mineral particles. Mycorrhizas can be instrumental in obtaining phosphorus from either source by increasing diffusion zones around roots and by the production of phosphatase enzymes. The stimulus for roots to produce phosphatase enzyme is a low availability of inorganic phosphorus in soil and a high demand by the root. The ability of an acidic organic soil to supply phosphate can not easily be determined by chemical analysis; thus a method for integrating root demand for soil supply of phosphorus has been developed using a root bioassay (Harrison and Helliwell 1979). The method has been successfully used to detect deficiencies in phosphorus of forest stands (Dighton and Harrison 1983, 1990).

As an initial assessment of the phosphorus relations of *R. maximum* in the Appalachians, we studied the nature of its mycorrhizal status in relation to the demand

Correspondence to: J. Dighton

for phosphorus by the roots and their ability to produce acid phosphatase. Temperature and rainfall vary with altitude and these factors affect the rate of litter turnover, mineralization and nutrient availability. This preliminary study was, therefore, conducted at three different altitudes.

Materials and methods

Site description

The Coweeta Hydrologic Laboratory is situated in the southern Appalachians near Franklin, North Carolina (35°04′ N, 83°25′ W). The climate is mesic with about 1800 mm of rainfall per year. A number of watersheds in the area are used as part of the Long-Term Ecological Research (LTER) Programme (Swank and Crossley 1988). The flora of native deciduous hardwood cove forest consists of *Quercus prinus* L., *Cornus florida* L., *Acer rubrum* L., and *Carya* spp. (Boring et al. 1988). As a result of gap formation, invasion by *R. maximum* has led to the establishment of extensive, dense, monospecific stands of this species. Three sites were selected under pure stands of *R. maximum* at different altitudes: Pickens Nose (PN) (1490 m), Watershed 27 (WS 27) (1160 m) and Watershed 18 (WS 18) (730 m). Soils range from inceptisols at higher elevations to ultisols at the lowest elevation.

Root sampling

Roots were collected for morphological and mycorrhizal characterization during the summer of 1990. Because the high organic content of the soils prevented root extraction by sieving, roots were hand-sorted from 4.5-cm-diameter cores taken to a depth of 10 cm from each site. Cores were taken from the centre of *R. maximum* stands and close to stems of the same species in order to avoid roots from any other tree species. The morphology and mycorrhizal associations of field-collected roots were compared to commercially grown *R. maximum* seedlings rooted in fertilized forest floor duff (John L Huffman, Native Plant Nurseries, Otto, N. C.). Roots were collected in August for phosphatase determinations and early September for estimates of phosphorus status, using the root bioassay.

Root morphology and mycorrhizal status

Roots were hand sorted from soil and examined fresh under a dissecting microscope to determine root morphological characters and colour. Samples of each type of root were preserved in 1.2% aqueous glutaraldehyde. Detailed examination of ectomycorrhizal structures was carried out according to the guidelines of Agerer (1987) and Ingleby et al. (1990). Root squashes were made in 0.1% trypan blue and mounted in lactophenol. Freehand transverse sections were stained in trypan blue and mounted in lactic acid and glycerol (50:50, v:v). These preparations were observed microscopically to characterize the nature of the sheath and extent of Hartig Net development. Other roots were cleared, decolorized and stained for vesicular arbuscular mycorrhizae (VAM) using acid fuchsin according to the method of Kormanik et al. (1980).

Root surface acid phosphatase activity

Root surface phosphatase activity was measured by a modification of the method described by Woolhouse (1969) on individual root pieces washed free of adhering soil particles and classified according to the three root classes. Each mycorrhizal morphotype was treated separately. Two ectomycorrhizal classes were identified, one consisting of *Cenococcum geophilum* Fr. mycorrhizas which occurred frequently, and the other a mixture of all other types of ectomycorrhiza. Roots were placed into glass tubes in 0.5 ml of 1 mM trisodium citrate buffer adjusted to pH 4.5. After the addition of 1.5 ml of 6 mM *p*-nitrophenol phosphate substrate the samples were incubated with intermittent shaking at 20° C for 30 min. Root pieces were removed and fixed in 1.2% glutaraldehyde for further study and the solution reacted with 5 ml 0.1 M NaOH to develop a colour reaction with the released *p*-nitrophenol. The coloured product was assayed colorimetrically at 410 nm and the concentration of *p*-nitrophenol released determined by calibration with standards.

The diameters of each root piece were measured using an eyepiece micrometer on a Zeiss microscope. Root segment length was measured either in the same way, or by direct measurement of longer pieces to the nearest 0.5 mm. Assuming the roots to be cylinders, the phosphatase activity for each root fragment was calculated per unit surface area. Hair-like and beaded roots were subsequently stained in acid fuchsin (Kormanik et al. 1980) to determine the nature and degree of the mycorrhizal association.

Assessment of plant phosphate status

Plant nutritional status was assessed using the phosphorus bioassay on roots (Harrison and Helliwell 1979; Dighton and Harrison 1983). This method relies on the influx of a ^{32}P-labelled phosphate solution (5×10^{-6} M) into excised roots. Radioactivity was measured in intact roots in water using Cerenkov radiation and corrected for colour and physical quench to give dpm values which were then translated to actual phosphate uptake as pg P mg^{-1} root calculated from the specific activity of the uptake solution. The influx is inversely related to the previous supply of phosphorus to the growing root, i.e. a high uptake value indicates a high demand for phosphorus due to poor availability. Individual ectomycorrhizal units (ECM) or short lengths of hair-like and beaded roots were placed in 1-cm-square nylon mesh bags (1-mm aperture) and the open end was closed around a small cardboard label with a staple. This method allowed the small root fragments to be adequately contained and labelled. The roots were then processed through the phosphorus bioassay (Harrison and Helliwell 1979).

Measures of soil phosphate status

Soils were divided into two horizons, an upper organic and a lower mineral zone. The depth of the organic horizon was greater in the higher altitude sites, as was the degree of incorporation of fine organic matter into the mineral horizon. Soil pH was measured in water, and soil extractable phosphate was determined using 60 ml Truog's extractant on approximately 2.5 g (fresh weight) soil samples (Allen et al. 1974). Results were corrected for initial soil weight and the wet weight to dry weight ratio, giving final values of μg P g^{-1} dry wt. of soil. The phosphate fixing capacity of the soils was determined according to the method of Bache and Williams (1971), the results being expressed as μg phosphate fixed g^{-1} dry wt. of soil. Data were analysed by standard ANOVA and General Linear Model (GLM) procedures of Statistical Analysis Systems, (SAS, SAS Institute Inc., SAS Circle, Cary, N.C.) and results are expressed as means with approximate standard errors. Where ANOVAs were significant, the significance of differences between individual means was calculated.

Results

Root morphology and mycorrhizas

Three root morphologies were found: (1) fine, hair-like roots consisting of a stele and a single layer of cortical cells which had an outer lattice of thick, brown hyphae;

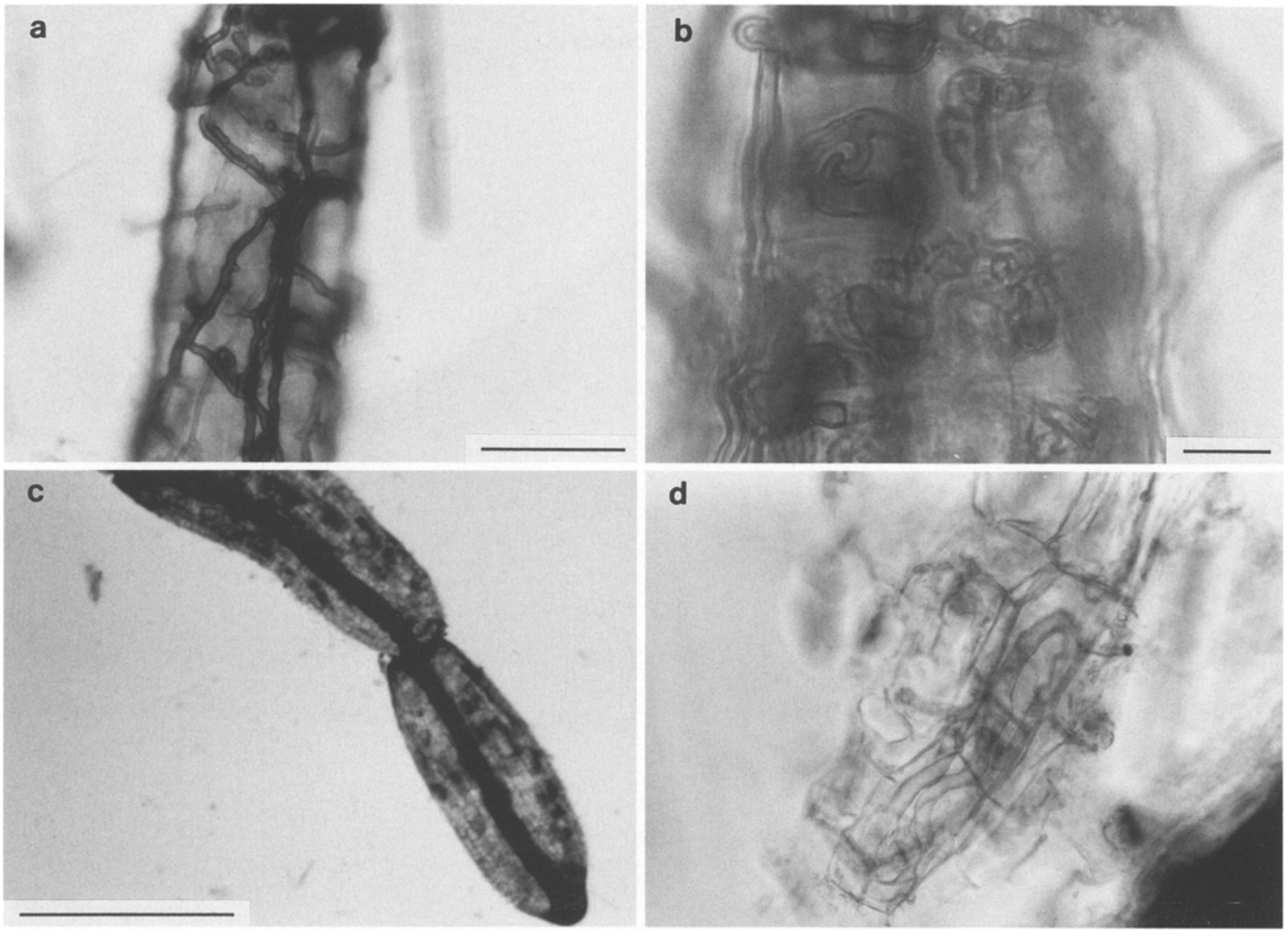

Fig. 1a–d. Characteristics of "lattice" infection of hair-like roots and beaded roots with VAM infection. **a** Lattice of thick-walled, brown mycorrhizal hyphae on the surface of a hair-like root; *bar* = 50 μm. **b** Hyphal coils formed by the "lattice" type of mycorrhiza in a hair-like root; *bar* = 10 μm. **c** Beaded root showing diameter constriction. Cortex contains VAM arbuscules; *bar* = 1 mm. **d** VAM hyphal coil/young arbuscule in a cortical cell displaced from a beaded root; *bar* = 50 μm

(2) beaded roots, containing a stele and a number of cortical cell layers; (3) roots which showed evidence of a fungal sheath. Mycorrhizas and root morphologies of different types were found on different parts of each root system, i.e. were not adjacent to each other on the same root segment.

Hair-like roots. These roots contained a central stele and a single layer of cortical cells. Some of these roots were covered with a lattice of thick-walled, brown hyphae of 3.5-μm diameter ("lattice" mycorrhizas). These hyphae branched frequently and contained numerous cross walls. The hyphae penetrated the cortical cells were they formed hyphal coils (Fig. 1). In addition, these roots contained intercellular arbuscules of large-diameter hyphae (5 μm), vesicles and occasional VAM spores. These VAM structures were less frequent than the infection by brown hyphae. Hair-like roots were common in nursery grown seedlings.

Beaded roots. Beaded roots were observed on both field-collected and nursery-grown *R. maximum*. The roots were 0.3–0.5 mm in diameter and regularly beaded (constricted) at about 1.5-mm intervals. The roots contained between 5–10 layers of cortical cells and produced abundant root hairs from the epidermis. Sparse VAM infection was observed where intercellular hyphae and arbuscules were observed (Fig. 1).

Ectomycorrhizal roots. These roots were identified by the presence of a fungal sheath, increased diameter from the subtending root, nature of branching, colour and presence of Hartig net. Typically, the mycorrhizas were subtended by roots of approximately 0.15-mm diameter. Descriptions of the types (Appendix; Figs. 2, 3) are based on their gross morphology and sheath and Hartig net characteristics following the terminology of Agerer (1987) and Ingleby et al. (1990). Only *Cenococcum* ectomycorrhizas were observed on nursery-grown seedlings.

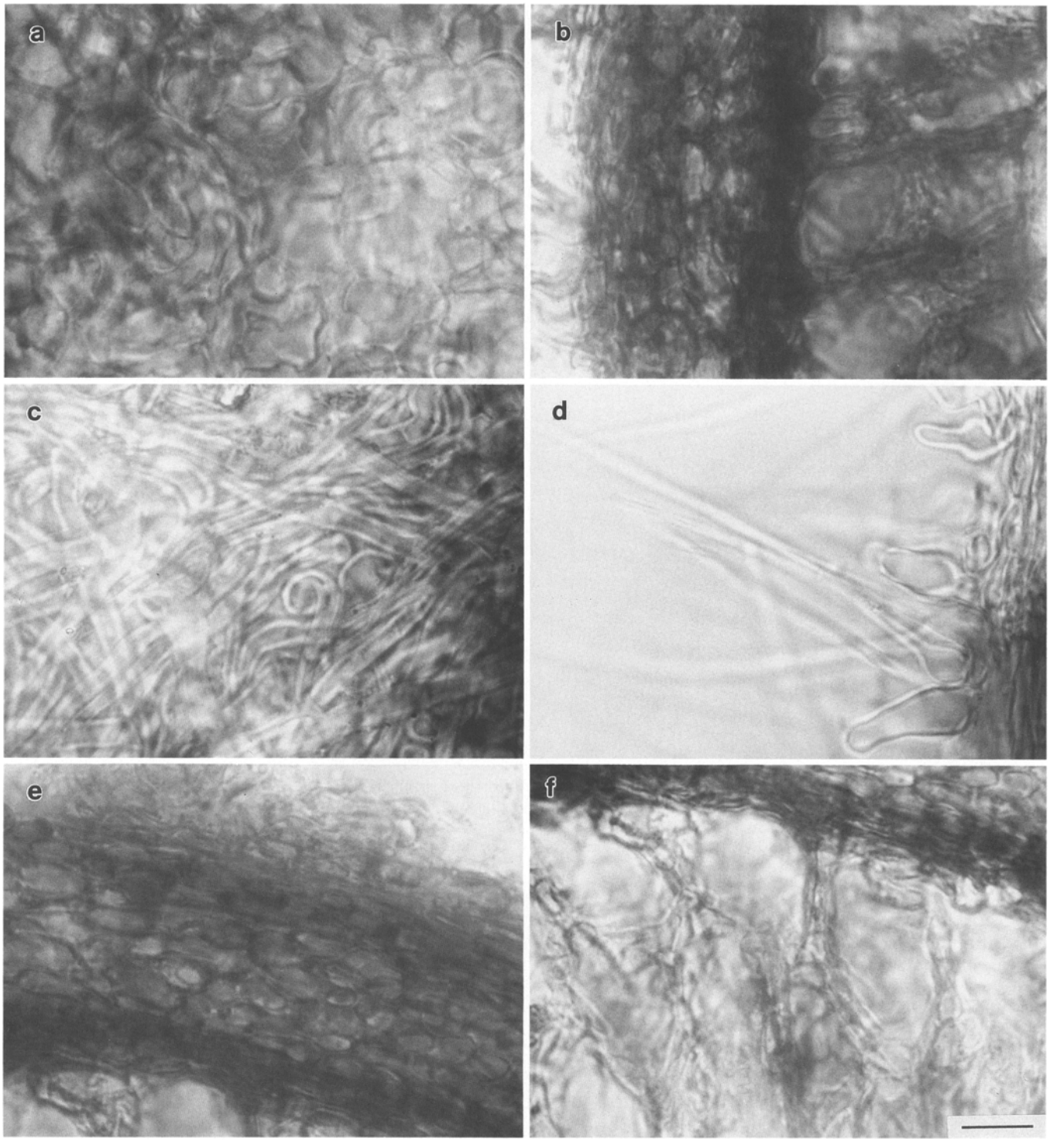

Fig. 2a–f. Characteristics of some of the ectomycorrhizas of *R. maximum*; *bar* = 10 μm. **a** Type *C*: outer sheath surface showing irregular-shaped hyphal cells forming the outer layers of the sheath (net synenchyma). **b** Type *C*: transverse section of root showing sheath structure, tanninized layer and Hartig net. **c** Type D: sheath surface showing filamentous hyphal construction of the prosenchymatous outer sheath and adpressed trichomes. **d** Type D: transverse section through the sheath showing outer prosenchymatous structure over a pseudoparenchymatous inner region. Note the characteristic trichomes and cystidia of the sheath surface. **e** Type E: transverse section of sheath showing pseudoparenchymatous construction underlying a net synenchyma giving rise to emergent hyphae. **f** Type E: transverse section of root showing Hartig net

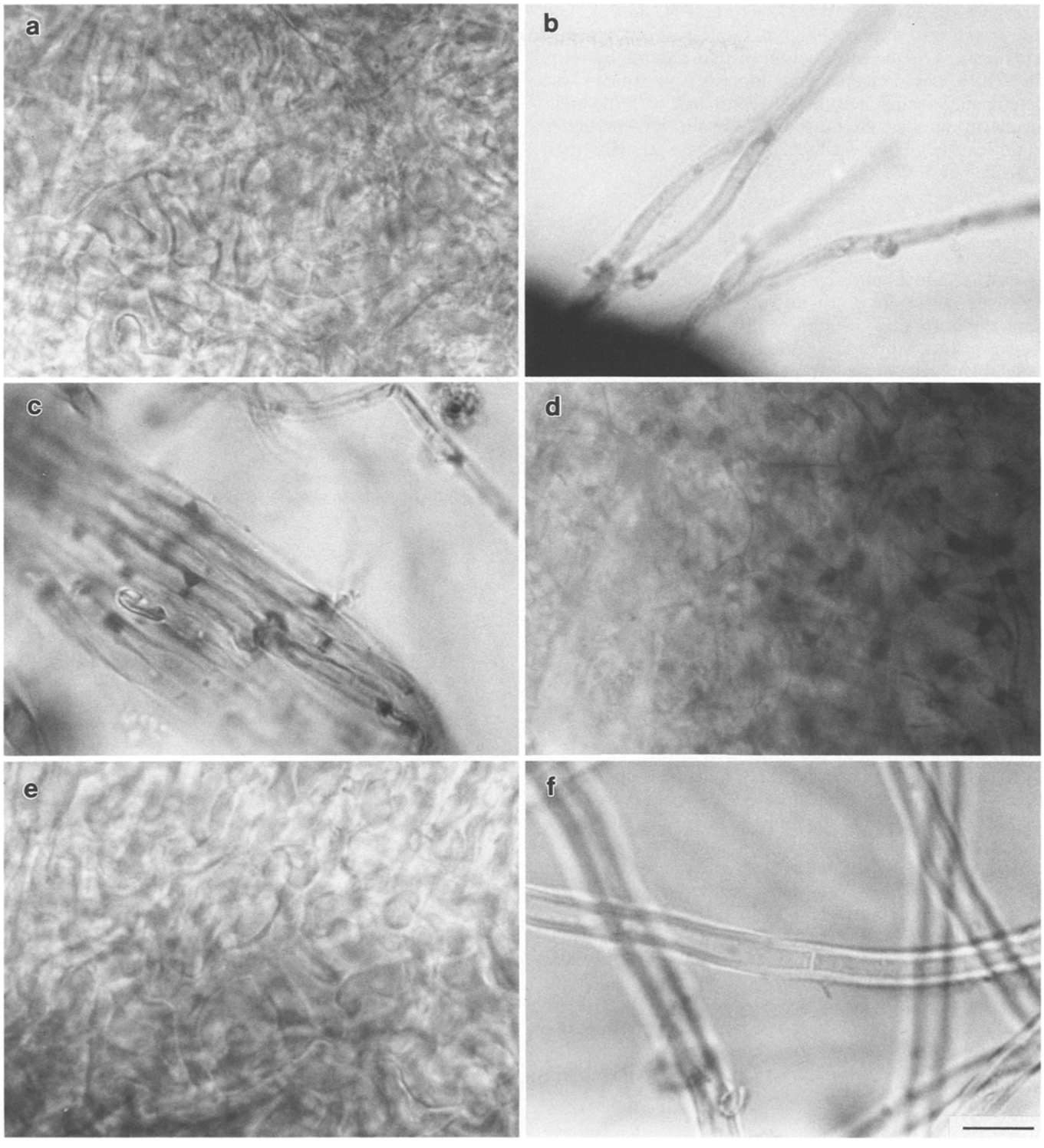

Fig. 3a–f. Characteristics of some of the ectomycorrhizas of *R. maximum*; *bar* = 10 μm. **a** Type F: sheath surface showing elongated hyphal cells of the outer net prosenchyma. **b** Type F: emergent clamp-bearing hyphae. **c** Type H: emergent hyphae forming a strand. **d** Type H: sheath showing surface filamentous hyphal construction of the net prosenchymatous outer sheath. **e** Type I: sheath surface showing loose organization of plectenchymatous sheath. **f** Type I: emergent thick-walled, wide-diameter hyphae showing one of the numerous cross walls

Root surface phosphatase activity

The results of root surface phosphatase activity are given in Table 1 and are analysed by the SAS GLM routine as a factorial analysis. It can be seen that the overall enzyme activity across all mycorrhizal types was significantly greater for the lower elevation site (WS 18). This was mainly due to the response of the "lattice" mycorrhizas, whose phosphatase activity was significantly higher at WS 18 than at the other two sites [means (±SE)

are: WS 18, 150±14; WS 27, 97±6; PN, 86±6], and the trend was similar, but not significant for ectomycorrhizas. A higher production of phosphatase by roots in WS 18 would suggest that inorganic phosphate was less readily available at this site compared with the higher elevation sites. Ectomycorrhizal root systems had a significantly higher surface phosphatase activity than "lattice" or VAM root morphologies (Table 1).

Estimate of available phosphate

Results of the Truog's extractable phosphate from soils from each site are given in Table 2. The data suggest that there were no differences in phosphate availability between sites. However, it is known that measures of extractable phosphate in low pH, organic soils are unreliable (Harrison 1987) and these results may not be truly indicative of the phosphate actually available to roots.

Root bioassay as an index of phosphate status

Results from the bioassay on roots from each site are presented in Table 3, analysed as a factorial design using the GLM routine in SAS. These results showed a significantly greater phosphate demand by *R. maximum* growing at the lower altitude site (WS 18) compared with the higher altitude sites. This would indicate that the soil from WS 18 was less able to supply inorganic phosphorus to the root (Harrison and Helliwell 1979). If the level of soil solution phosphate were low, negative feedback (Kroehler et al. 1988) would induce the higher phosphatase activity of the roots seen at this site.

There were no significant differences in P uptake between mycorrhizal types, although from the phosphate uptake data presented in Table 3, there was an indication that beaded roots had better phosphate nutrition (lower P uptake) than roots with other morphologies. Beaded roots, however, only regularly occurred at WS 27, never at WS 18 and only on some sampling occasions at PN. A detailed study of the spatial distribution of different types of root and mycorrhizal associations on the root system was not carried out, and quantitative determination of the relative abundance of the three root morphotypes was not possible because quantitative extraction of the fine hair-like roots from the highly organic soil was impractical.

Phosphate-fixing capacity of soils

Determinations of the P-fixing capacity of the soils from the three different altitudes are given in Table 4. In all cases, the fixing capacity was higher in the mineral component of the soil than the organic component. P fixation at the PN (high altitude) site was significantly less than at the two lower-altitude sites. These data suggest that phosphate was less readily available at lower altitudes due to changes in soil chemistry and probably due also to a reduction in the organic component. These data fit with the root bioassay P-uptake data showing that roots from both lower-altitude sites had a greater phosphate demand than at PN (although only the uptake at WS 18 was statistically significantly greater) and the fact that root surface phosphatase activity was greatest at WS 18.

Table 1. Mean root surface phosphatase activity (ng *p*-nitrophenol released per mm^{-2} root surface area ± SE) of *R. maximum* with different root morphologies. Means followed by a different letter are significantly different at the 5% level by Tukey's Honestly Significant Difference Test. The interactions site/root morphology ($F=0.02$) and site/mycorrhiza ($F=0.12$) are not statistically significant

Between sites: ($F=4.56$; $df=2,82$; $P<0.05$; ANOVA)		
Pickens Nose (1490 m)	249±39 ab	($n=30$)
Watershed 27 (1160 m)	224±28 a	($n=28$)
Watershed 18 (730 m)	383±62 b	($n=32$)
Between root morphologies: ($F=18.99$; $df=2,82$; $P<0.001$; ANOVA)		
Hair-like roots	116± 8 a	($n=32$)
Beaded roots	202±14 a	($n=15$)
Ectomycorrhizal roots	448±46 b	($n=43$)
Between mycorrhizal types: ($F=12,16$; $df=3,82$; $P<0.001$; ANOVA)		
Lattice roots	106±10 a	($n=23$)
Vesicular-arbuscular	179±13 a	($n=24$)
Ectomycorrhizal (*Cenococcum*)	401±27 b	($n=13$)
Ectomycorrhizal (others, mixed)	468±65 b	($n=30$)

Table 2. Truog's extractable phosphate (mean±SE) from soils collected from each site under *R. maximum* (mean of 6 replicates). From ANOVA (SAS, GLM), the difference between sites ($F=0.13$; $df=2,29$) and between the organic and mineral horizons ($F=0.86$; $df=1,29$) is not statistically different

Site	µg P g^{-1} dry wt. of soil	
	Organic	Mineral
Pickens Nose	27.2± 4.0	32.0±1.8
Watershed 27	30.1± 1.7	27.7±2.5
Watershed 18	38.8±13.7	24.3±2.4

Table 3. Uptake of phosphorus into roots of *R. maximum* as an index of phosphate demand (mean±SE). Means followed by different letters are significantly different at the 5% level by Tukey's Honestly Significant Difference Test. The interaction site/root morphology ($F=0.05$) is not statistically significant

Between sites: ($F=5.16$; $df=2,83$; $P<0.01$; ANOVA)		
	pg P mg^{-1} root	
Pickens Nose	1434±132 a	($n=30$)
Watershed 27	1926±197 ab	($n=30$)
Watershed 18	2309±271 b	($n=30$)
Between root morphologies: ($F=2.05$; $df=2,83$; P NS; ANOVA)		
Beaded	1332±115	($n=10$)
Ectomycorrhizal	1955±202	($n=40$)
Hair-like	1964±189	($n=40$)

Table 4. Phosphate fixing capacity of soils under *R. maximum* (means ± SE). Means followed by different letters are significantly different at the 5% level by Tukey's Honestly Significant Difference Test. From ANOVA, the differences between sites are significant ($F=14.38$; $df=2,12$; $P<0.001$), differences between horizons are not significant ($F=3.72$; $df=1,12$; $P<0.078$), neither is the interaction site/horizon ($F=0.07$; $df=2,12$; P NS)

Site		P fixation ($\mu g\ PO_4\ g^{-1}$ soil)		
Pickens Nose	(organic)	95 ± 216		
	(mineral)	365 ± 244	230 ± 158 a	($n=6$)
Watershed 27	(organic)	968 ± 113		
	(mineral)	1157 ± 38	1062 ± 68.2 b	($n=6$)
Watershed 18	(organic)	698 ± 181		
	(mineral)	1000 ± 54	848 ± 108 b	($n=6$)

Discussion

Previous investigations of the mycorrhizal associations of *Rhododendron* species indicated that the genus can associate with a number of mycorrhizal fungal partners. The main mycorrhizal type appears to be ericoid (Pearson and Read 1973; Read and Stribley 1975; Peterson et al. 1980; Duddridge and Read 1982). The isolation of the ericoid endophyte *Hymenoscyphus ericae* (Read) Korf and Kernan from *Calluna vulgaris* (L.) Hull (Pearson and Read 1973) provided a mycorrhizal fungus which has been subsequently used for mycorrhizal synthesis and experimentation with *Rhododendron* spp. (Moore-Pankhurst and Englander 1981; Bradley et al. 1982; Duddridge and Read 1982).

Seviour et al. (1973) described the ericoid mycorrhizal association of both *Azelia indica* L. and *Rhododendron* spp. with the basidiomycete *Clavaria* spp. This association has been implicated in carbon and phosphate flux between *Rhododendron* and *Pieris japonica* D Don. through possible mycorrhizal linkages between their roots (Englander and Hull 1980). Largent et al. (1980) also described arbutoid and ectomycorrhizal roots in their samples from *R. macrophyllum* D. Don. and *R. occidentale* (T and G) Gray from northern California.

VAM have been shown to produce infection rates of 50–60% in *R. simsii* Planch roots inoculated with the VAM fungi *Glomus faciculatum* (Thaxter sensu Gerd) and *G. mosseae* (Nichol. and Gerd). This infection significantly enhanced both shoot and root growth. In his description of *R. ponticum* L. in the British Isles, Cross (1975) states that its mycorrhizal flora includes VAM. VAM are also known to infect thicker (> 1.5 mm diameter) roots of *Vaccinium* and *Styphelia* species in Hawaii (Koske et al. 1990).

In this study we have shown that *R. maximum* in the southern Appalachians forms VAM and ectomycorrhizal associations with differing root morphologies. The relative proportions of the three root morphologies in the total root population could not be determined as it was not possible to quantitatively extract the fine, hair-like roots from the highly organic soils. As phosphate is a limiting factor for plant growth, the ability of the differing root morphologies and mycorrhizal types to obtain phosphate were investigated in relation to altitude.

Acid phosphatase activity was shown to be greatest at the lowest altitude site and in the ectomycorrhizal roots. Two hypotheses can be suggested for the greater activity in ectomycorrhizal associations. Firstly, ectomycorrhizal fungi have a greater inherent capacity to produce phosphatase enzymes or, secondly, as a result of the higher hyphal component of the ectomycorrhizal sheath, there are more microsites for phosphatase-producing rhizospheric microorganisms than on the roots of the other mycorrhizal associations. Neither of these hypotheses has been tested here, but the data suggest that the ectomycorrhizal root system is able to obtain phosphorus from organic complexes in soil to a greater extent than the other mycorrhizal types associated with *R. maximum*. Surface phosphatase activity of *Cenococcum* mycorrhizas was lower but not significantly different from other ectomycorrhiza types. Because of the low numbers of roots of each mycorrhizal category, it was not possible to look at the effect of each mycorrhizal type on phosphatase production.

The ability of mycorrhizas to produce phosphatase enzymes is probably linked to the availability of soluble phosphorus in the soil. Truog's extractable phosphate is a measure of the instantaneous soil solution pool and is often considered to be that pool "available" to plant roots. Results from this study indicated that the size of this pool as determined by this method did not vary between the sites. It is known, however, that chemical determination of phosphate availability in organic soils is not very reliable. The root phosphate bioassay (Harrison and Helliwell 1979; Dighton and Harrison 1983, 1990) is thought to be a better index of plant-available phosphate than chemical analyses; it integrates the demand for phosphate by the plant and the phosphate-supplying capacity of the soil. From the root-bioassay data of phosphate uptake, it was shown that trees growing at the lower altitudes had a greater demand for phosphate than at the highest altitude probably indicating lower phosphate availability at the lower altitude. This in turn correlates well with the increased root phosphatase activity at the lower altitude sites which may have been induced by the limiting supplies of the inorganic form of the element (Kroehler et al. 1988).

In general the availability of nutrients via mineralization of organic resources increases as edaphic factors become more favourable. At high altitudes, mineralization is often limited by high rainfall and low temperatures; conditions at lower altitudes are usually more favourable. Therefore phosphate should be more available at low altitudes and the roots consequently less deficient, producing less phosphatase enzyme. The phosphate-fixing capacity of these soils, however, increase with decreasing altitude. Thus, where conditions for mineralization improve, the released phosphate becomes more tightly bound to the soil by physico-chemical factors; plants at the lower altitudes become more deficient and roots are stimulated to produce phosphatase enzymes.

Although we have not been able to quantitatively determine the relative proportions of the three root mor-

photypes of *R. maximum*, we have shown some differences in their physiology. Ectomycorrhizal roots appear to be capable of producing significantly more surface phosphatase than either VAM or ericaceous mycorrhizal roots. Between the frequently occurring *Cenococcum geophilum* and other ectomycorrhizal types there were no significant differences in surface phosphatase activity within the ectomycorrhizal roots. Although not statistically significant, beaded roots appeared to be less phosphate deficient than ectomycorrhizal or hair-like roots. The fact that beaded roots were not found at all sites suggests that their occurrence may be related to other, as yet undetermined, soil characteristics.

We feel that we have progressed some way towards testing the hypothesis proposed by Largent et al. (1980) that soil factors are important in determining the nature of the mycorrhizal association of *Rhododendron* species. This has been examined here for only one species at one site. The results indicate the importance of looking at the relative contribution of different mycorrhizal associations on plant species which may associate with more than one mycorrhizal type. In this case, we can speculate that the species' fitness is enhanced by association with a range of mycorrhizal partners when the physiological capabilities of the different associations maximize nutrient availability under different soil conditions. The physiological differences between associations and the influence of soil factors on these physiological processes have not received sufficient attention in the past.

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Appendix. Description of the morphological and structural characteristics of ectomycorrhizas of *R. maximum* at Coweeta

Type A

Morphology. Elongate, pinnately branching, cream ageing to rufous-brown-coloured ectomycorrhizas. Terminal root tip up to 5 mm in length with one to many side branches with a diameter of 0.2–0.3 mm. The sheath had a smooth surface and no obvious emergent hyphae.

Root squash. The sheath surface was composed of an outer layer of highly branched, net, prosenchymatous hyphal cells over an inner layer of almost globular, regular synenchyma. Few emergent hyphae were seen.

Root section. The sheath was about 25 μm thick, consisting of 6–8 layers of fungal cells, with the outer layers of cells becoming plectenchymatous and elongate around the circumference of the root. The Hartig net was obvious and extended to the base of the outer layer of cortical cells.

Type B

Morphology. Monopodal or occasionally branching, black mycorrhizas with extensive production of thick, black, emergent hyphae. Mycorrhizal units (aggregates of root tips of the same mycorrhizal type) were up to 2 mm in length, consisting of terminal roots of length 0.7–1 mm and diameter 0.3 mm. These were identified as *Cenococcum geophilum* mycorrhizas.

Root squash. The sheath surface consisted of tightly packed, net synenchymatous fungal cells.

Root section. The sheath consisted of only a few (5–6) layers of hyphae and was about 15–25 μm thick. The Hartig net was obvious, penetrating between the outer cortical cell layer. Melanization of the hyphae of this species made observation of structures somewhat difficult.

Type C

Morphology. Monopodal, creamy to rufous-brown mycorrhizas emerging directly as a branch from the subtending root, or as a terminal feature of a short root. The length of the mycorrhiza was typically 1 mm with a diameter of about 0.3 mm. The sheath surface was smooth and slightly shiny with no emergent hyphae.

Root squash. The sheath surface consisted of net synenchymatous fungal cells overlying irregular, non-interlocking synenchyma (Fig. 2a). In some roots the basal part of the mycorrhiza had a sheath with a less filamentous structure than that of the distal portion.

Root section. The sheath was some 20–30 μm thick and composed of 10–15 layers of fungal cells. The sheath cells were cuboidal or slightly flattened and pseudoparenchymatous in nature. The Hartig net was obvious between the outer layer of cortical cells (Fig. 2b). Emergent hyphae were not seen.

Type D

Morphology. Pinnate to loosely coralloid, thick, creamish-white mycorrhizas. Mycorrhizal units were up to 5 mm long with numerous side branches. Mycorrhizal diameter varied from 0.5 to 0.7 mm. The sheath surface occasionally bore patches of yellow colour and short, emergent hyphae caused adherence of soil particles.

Root squash. Sheath surface consisted of a mixture of bulbous-based trichomes of 70–80 μm in length and 2.5 μm in diameter and squat-shaped cystidia with swollen bases of 25 μm length and basal diameter of 10 μm (Fig. 2c, d). This outer layer gave the appearance of being a felt prosenchyma overlying an inner sheath of non-interlocking synenchyma of globose cells.

Root section. The sheath in section was some 25–30 μm thick and consisted of 15–20 layers of fungal cells elongated in the axis of the root circumference. The epidermal region of the root was heavily tanninized. A Hartig net was present.

Type E

Morphology. A three-dimensionally branched (but not truly coralloid), thick, creamy-coloured mycorrhiza. Terminal mycorrhizal units extended up to 2.5 mm in length with mycorrhizal, short roots being of 0.3 mm di-

ameter. The sheath surface was dull and covered with emergent hyphae.

Root squash. The sheath surface consisted of irregularly shaped hyphal cells forming a net synenchyma overlying an interlocking, irregular synenchyma.

Root section. The sheath was some 40 µm thick and consisted of 10–15 layers of pseudoparenchymatous hyphal cells. The emergent hyphae were 110 µm long and about 5 µm in diameter without a swollen base. The Hartig net was obvious and extended between the outer layer of cortical cells.

Type F

Morphology. Elongate pinnate to loosely coralloid, thick light-creamy-coloured mycorrhiza with loose wefts of hyaline, emergent hyphae. Mycorrhizal clusters were up to 3 × 2 mm in extent with root diameters of 0.3–0.4 mm. The sheath surface had a clear covering of hyaline hyphae giving it an almost gelatinous appearance (Fig. 3a).

Root squash. The sheath surface consisted of elongate- to tetrahedral-shaped fungal cells (outer net prosenchyma overlying a net synenchyma) giving rise to emergent hyphae of 2.5 µm diameter and bearing clamp connections (Fig. 3b).

Root section. The sheath was extensive, consisting of some 10–15 large hyphal cells and was 50 µm thick. The Hartig net was indistinct and penetrated between the outermost layer of cortical cells only.

Type G

Morphology. Smooth pinnate to irregularly branched, grey-coloured mycorrhizas. The branching was varied in degree, forming either units of root clusters of 2 × 4 mm or individual monopodal or bifurcate root tips 2 mm in length. The average diameter of the mycorrhizas was 0.4–0.5 mm. The sheath appeared grey with a somewhat greasy appearance.

Root squash. The sheath surface consisted of an outer net synenchyma with frequent cross walls overlying a non-interlocking, irregular synenchyma whose cells became almost tetrahedral in shape.

Root section. The sheath consisted of 10–15 layers of pseudoparenchymatous cells which were almost cuboidal in section. The sheath was about 30 µm thick. The Hartig net was obvious, penetrating between the outer two layers of cortical cells.

Type H

Morphology. Short monopodal or bifurcate, light-creamy-yellow-coloured mycorrhizas with extensive, hyaline, emergent hyphae coalescing to form rhizomorphs (Fig. 3c). The mycorrhizal units were at most 2 mm in length and 0.3 mm in diameter.

Root squash. The sheath surface consisted of a distinct layer of hyphae with frequent cross walls forming a net prosenchyma (Fig. 3d). Beneath this outer layer was an interlocking, irregular synenchyma of more globular cells.

Root section. The sheath wall in cross-section consisted of 15–20 plectenchymatous, elongate cells. The Hartig net was distinct between the outer two layers of cortical cells.

Type I

Morphology. Monopodal, creamy-brown-coloured mycorrhizas with distinct hyaline, emergent hyphae. Mycorrhizal lengths varied from 0.5 to 1 mm with a diameter of about 0.3 mm.

Root squash. The outer sheath consisted of a disorganized layer of net plectenchyma giving rise to emergent hyphae (Fig. 3e). This overlaid a net synenchymatous layer of cells. The emergent hyphae were smooth-walled with occasional branching. They were about 7–8 µm diameter with thick walls and no visible clamp connections (Fig. 3f).

Root section. The sheath consisted of some 7–10 layers of globose to elongate, plectenchymatous cells. The Hartig net was obvious in the outer layer of cortical cells.