The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi

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Summary

• Here we present the ultrastructure and molecular identification of the fungi in the mycorrhizal nodules of four species of Podocarpaceae endemic to New Zealand. Podocarps form a major component of the rain forest of New Zealand, where they grow on soils poor in extractable phosphate and high in organic matter.
• Histological studies showed that the mycorrhizal nodules develop as modified lateral roots, and are colonised by an endophytic fungus. Scanning and transmission electron microscopy showed the fungus to contain intracellular coils and arbuscules typical of the order Glomales.
• DNA was extracted and amplified using PCR to identify mycorrhizal fungi. Several different lineages of arbuscular mycorrhizal fungi colonising the nodules were identified. Individual nodules contained more than one fungal lineage.
• This study is the first to indicate species of Glomales present in the New Zealand rain forest. It is likely that many of the taxa sampled are new to science because there has been little taxonomic work on Australasian Glomales.

Key words: Arbuscular mycorrhiza, Podocarpaceae, mycorrhizal nodule, Glomales.


Introduction

The Podocarpaceae is a predominantly southern hemisphere gymnosperm family of which 17 species (belonging to eight genera) are endemic to New Zealand. The family has a fossil record extending back to the Triassic. Podocarps are an important component of the indigenous rain forests of New Zealand, which have an ancestry stretching back to the ancient southern continent of Gondwana 190 million yr ago (Salmon, 1980). The soils of these rain forests are extremely low in available phosphate and often waterlogged. The ability of the Podocarpaceae to thrive in these conditions is considered to be due to their symbiosis with mycorrhizal fungi, which inhabit the nodules on their roots. Baylis et al. (1963) found that fungal infection was necessary to sustain the growth of podocarp seedlings in natural soils. The lowland podocarp forests have been extensively logged, but large areas of primary forest remain. These areas are under severe pressure from introduced herbivores.

The roots of the three ancient gymnosperm families Araucariaceae, Podocarpaceae, and Phyllocladaceae are known to be covered in small protuberances, or nodules (McLuckie, 1923; Yeates, 1924; Saxton, 1930). A study of the roots of the Podocarpaceae in New Zealand by Baylis et al. (1963) showed them to be studded with closely spaced nodules forming between two and four longitudinal rows along the length of the root. Both their small and uniform size and mode of development, distinguish them from the N2-fixing nodules of legumes and actinorhizal plants. Early studies described the existence of a nonseptate filamentous fungus in the cortex of the nodules, leading to the designation of mycorrhizal nodules. Baylis et al. (1963) described a phymycetous fungus bearing vesicles and arbuscules indistinguishable from that occurring in the roots of the forest dicotyledons in New Zealand. Morrison & English (1967) considered Podocarpaceae nodules to be colonised by a member of the genus Endogone. Currently, all root fungal endophytes bearing arbuscules are placed in the order Glomales (or Glomerales), a group of obligate symbiotic fungi associated with the roots of the vast majority of vascular plants. Comparable mycorrhizal nodules have been described in just one other family of tropical trees, the angiosperm family Casuarinaceae (Duhoux et al., 2001). In two Gymnostoma species belonging to this
family, the fungi infecting the mycorrhizal nodules have been identified as belonging to the Glomales. Unlike podocarps, the Casuarinaceae root systems have both mycorrhizal nodules and N₂-fixing actinorhizal nodules.

Arbuscular mycorrhizal (AM) fungi are by far the most widespread mycorrhizal symbionts in New Zealand's forests. The fungal mycelium penetrates the cortex of the root to produce typical intracellular structures such as arbuscules and coils. Johnson (1977) found that most vascular plants in New Zealand's coniferous-dicotyledonous forests harboured typical arbuscular endophytes. However, the species of arbuscular fungi present in the forests were identified by the collection of spores in the soil (Hall, 1977; Johnson, 1977). Spore counts alone do not necessarily correlate with the diversity of species colonising plant roots (Helgason et al., 1999). Also some species of mycorrhizal fungi may not form spores. Hall (1977) working in the New Zealand Catlin forest found that there was no correlation between soil spore numbers and infection levels in adjacent hosts. When only hyphal structures are present, it is generally only possible to identify fungi to the level of family or genus (Merryweather & Fitter, 1998). In addition, members of some families cannot be detected by standard stains (Morton & Redecker, 2001). The diversity of AM fungi present in a community can directly affect plant diversity (Van der Heijden et al., 1998). For a full understanding of the ecology of plant–fungus interactions in natural ecosystems it is necessary to be able to identify the individual species of AM fungi which are present.

Comparative studies of the nuclear ribosomal RNA genes have made possible the identification of arbuscular mycorrhizal fungi in planta (Bruns et al., 1991; Simon et al., 1992). While most studies have used known species of fungi in glasshouse pot cultures, studies under field conditions have demonstrated the potential of these methods for analysing the taxonomic diversity of fungi actively colonising roots (Clapp et al., 1995; Helgason et al., 1999). We were interested in the identity of the fungi in podocarp mycorrhizal nodules as this would give a first indication of species of mycorrhizal fungi prevalent in New Zealand's west coast rainforests. We were also interested in asking questions such as whether different nodules on the same tree contained different lineages of colonising fungi, and whether individual nodules contained more than one fungal lineage.

In this paper we present the first study of the ultrastructure of podocarp mycorrhizal nodules and the identity of the fungal endophytes present in them using electron microscopy and molecular methods. Four podocarp species endemic to New Zealand were sampled: Prumnopitys ferruginea, P. taxifolia, Dacrycarpus dacyrioides, and Dacrydium cupressinum.

Materials and Methods

Two areas of primary rain forest on the west coast of New Zealand's South Island were chosen for the collection of plant material. The Okarito forest is dominated by 300-yr-old rimu trees (Dacrydium cupressinum), but a number of other conifers and broad-leaved species are also present, including miro (Prumnopitys ferruginea). The Poerua forest is dominated by pure stands of kahikatea (Dacrycarpus dacyrioides) of a similar age. Both forest sites are likely to have been disturbed by significant gravel outwashes following the ruptures on the South Island Alpine Fault, which occurred in 1420, 1620 and 1717. Some strands on the higher glacial moraines will have been undisturbed since the retreat of the last ice advance approximately 10 000 yr ago. The soils are highly acidic (pH 3.8–4.4) with high organic matter content, medium levels of nitrogen and low acid-extractable phosphorus. Annual rainfall is c. 3 600 mm evenly distributed throughout the year, frequently producing a high water table resulting in waterlogging of the soil. The climate is temperate oceanic, with a mean annual temperature of 11.3°C. Matai (Prumnopitys taxifolia) was sampled from nearby lowland montane forest.

In order to be certain of the species to which the sampled root nodules belonged, young (4–12 yr old) podocarp plants with nodulated roots were collected from the two study sites and maintained in their substrate for 24–48 h until return to the laboratory. Roots bearing nodules were carefully washed free of soil before fixing for histological studies. Twenty seedlings (> 1 yr) of Dacrycarpus dacyrioides with a single tap root and few or no existing nodules were also collected and planted in a steam-sterilised potting mix consisting of 2/3 sand and 1/3 vermiculite. Seedlings were grown in an unheated glasshouse under natural light. Cuttings of a related podocarp planted in the same soil mix produced root nodules after 4 months. The nodules were entirely free of fungal infection.

Microscopical analyses

Mycorrhizal nodules collected in the field were fixed overnight in 2% paraformaldehyde, 1% glutaraldehyde, and 0.5 tannic acid, in 0.075 M sodium phosphate buffer at pH 7.2. Following several rinses in buffer, the specimen were postfixed in 1% osmium tetroxide for 3 h, dehydrated in a graded ethanol series and embedded in Spur's resin for transmission electron microscopy or transferred to amyl-acetate and critical point dried for scanning electron microscopy. One µm thick sections were cut with a microtome and stained with 0.5% (w/v) toluidine blue for examination with a light microscope. For TEM, resin blocks were thin-sectioned with a diamond knife and sequentially stained with 2% aqueous uranyl acetate for 10 min and lead citrate for 5 min. Observations were made with a JEOL-1200EX electron microscope at 60 kV.

Molecular methods

Genomic DNA was extracted from five root nodules from each of the four species (Prumnopitys ferruginea, P. taxifolia,
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**Dacrycarpus dacrydioides**, and **Dacrydium cupressoides**. The root nodules were washed several times in dH2O and sonicated for 1 min at a time between washes in order to remove soil sticking to the nodule surface. Preliminary data suggested that, without extensive cleaning, Ascomycete and Basidiomycete soil fungi adhering to the outer nodule surface were preferentially amplified via the polymerase chain reaction (PCR). Two root fragments from each species (c. 2 mm long) adjacent to root nodules were also extracted. Single lyophilized root nodules excised from the root axis with fine tweezers or root fragments were extracted in 300 µl of CTAB buffer using methods described by Gardes and Bruns (1993). However instead of precipitating the DNA, we added 16 µl of Glassmilk (BIO101, Inc., Carlsbad, CA, USA) and followed procedures outlined in the GeneClean II kit (BIO101, Inc.). We used PCR protocols described by Redecker (2000), universal primers from White et al. (1990) and Gardes & Bruns (1993), and new Glomales-specific primers as well as those from Redecker (2000) to amplify the nuclear small subunit (18s) and internal transcribed spacers (ITS). The 18s and ITS are the best represented regions of fungal rDNA, we designed two primers using sequence data from Redecker et al. (2000) and new sequence data obtained from PCR reactions nested in the universal primer set NS5/ITS4 with the Glomaceae- and Archaeosporaceae-specific primers. In the first amplification reaction for nested PCR, we used 2-min extension times. One new primer was designed for Glomus (GLO1375R: acttccatcggttaaacacc) and one for Archaeospora (ARCH1375R: tcaaacttccgttggctartcgcrc). For primer combinations where more than two root nodules were preferentially amplified via the polymerase chain reaction (PCR), we used restriction fragment length polymorphisms with the endonuclease Hinfl (New England Biolabs, Inc., Beverly, MA, USA) to select amplicons with unique restriction patterns for direct sequencing; otherwise, all amplicons were sequenced. PCR products were purified with a QiAquick kit (QiAGEN, Inc., Valencia, CA, USA), sequenced with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Biosystems, Foster City, CA, USA), and electrophoresed on an ABI 377 DNA Sequencer (PE Biosystems). We used DNA Sequencing Analysis v.3.4.1 and Sequence Navigator v.1.0.1 (PE Biosystems) to process raw data. The DNA sequences were used to query GenBank via BLAST. The nearest relatives of each Glomalean sequence were inferred with the neighbor-joining algorithm implemented in the program PAUP*4.0b8 (Swofford, 2001) from the DNA sequence alignment used in Redecker et al. (2000) with 255 of 1831 positions excluded from the analysis.

**Results**

**Mycorrhizal nodules**

All four species of podocarp in this study produced numerous spherical nodules, arranged in two to four close rows along the length of the roots (Fig. 2a). New nodules appeared continually throughout the year on older roots as well as on young lateral roots so most roots contained older nodules interspersed with young nodules (Fig. 2b). Nodules of *Pseudopanax taxifolia* were covered in multicellular root trichomes (Fig. 2b), as was a small proportion of *P. ferruginea* nodules. Neither of the other two species formed trichomes on the nodule epidermis (Fig. 2c). Young nodules were white with a soft epidermis, and became orange as they matured. The oldest nodules were dark brown with an epidermis consisting of several layers of compressed thick-walled dead cells.

Podocarp root cuttings in a sterile nutrient-free potting mix formed nodules after 4 months with no trace of fungal infection. Our observations confirm those of Baylis et al. (1963) and Kahn (1967) who found that seedlings of podocarp species raised in sterile culture formed typical nodules, but that the nodules did not contain any trace of endophyte. Nodules would therefore appear to be a constitutive feature of the root system.

Fungi could not be detected in young white nodules on seedlings collected from the field. Over a period of 6 months the kahikatea seedlings replanted in sterile potting mix continued to flourish and produce lateral roots and many nodules. Fungal hyphae were present in the long roots, and older nodules. Young nodules however, were without trace of fungal infection, despite the presence of hyphae in the adjacent root cortex.

In all four species studied the nodules originate in the root pericycle adjacent to a xylem pole, in a pattern similar to lateral root formation. Longitudinal sections (Fig. 3a) show each nodule to consist of an outer epidermis surrounding a cortex with an endodermis surrounding the central vascular system. The vascular strand of the developing nodule is connected to that of the main root, and usually traverses only half the length of the nodule. During the early period of growth, cortical cells appear to enlarge evenly throughout the nodule. The endodermis of the nodule forms a continuous boundary
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around the vascular system from the beginning of its development, unlike the open-ended endodermis of a developing lateral root. In this respect the mycorrhizal nodules of the podocarps appear to differ from those of the Casuarinaceae (Duhoux et al., 2001). There is no root cap. According to Kahn (1967) these features suggest that the nodules are unique morphological features, rather than modified arrested lateral roots. Also in contrast to root nodules of the Casuarinaceae, there is no obvious meristematic area at the distal end of the nodule.

Fungal structures

Our studies with the scanning electron microscope show clearly that the nodule cortex is colonised by a fungus with structures typical of an arbuscular mycorrhizal fungus. In Fig. 4(a) the formation of small highly branched arbuscules from the main trunk hyphae can be clearly seen. Vesicles can also be seen in some cells (Fig. 4b). The fungus colonises the root nodules as intracellular hyphae. Under the electron microscope, the hyphae can be clearly seen burrowing straight into the epidermal cell walls (Fig. 5a,b). The presence of heavy deposits of tannins in these cells, shown as the darkly staining material in the transmission electron micrographs, does not appear to be a deterrent to fungal hyphae penetrating into the nodule from the surrounding environment. Young nodules are soft, transparent and free of fungus. There is some evidence of mucilage surrounding them, which may serve as a means of adhering the fungal hyphae.

While the roots on which the nodules form have fungi in the cortical cells, these hyphae do not cross the endodermis into the vascular system. During the early development of the nodule, as the endodermal cells divide to form the new nodule epidermis, this barrier to fungal entry from the root cortex appears to remain.

Before their colonisation by fungi, the nodule cortical cells contain high amounts of starch and large nuclei (Fig. 5c). In heavily colonised cells there is no evidence of starch. Cells containing the fungus show a varying degree of cytoplasmic content, depending on the degree of colonisation (Fig. 6a,b). In older nodules there is no evidence of cytoplasm and the cortical cells are packed with fungal structures.

In mature nodules the fungus colonises the cortical tissue throughout the nodule, with the exception of cells adjacent to

Fig. 2 Mycorrhizal root nodules of podocarps. (a) Podocarp root system bearing nodules. (b) Root of Prumnopitys taxifolia showing new nodules developing on an older root and older nodules covered with trichomes. (c) Root system of Dacrydium dacrycarpus bearing mycorrhizal nodules. In this species new nodules develop from the endodermis of the old nodule. (d) Scanning electron micrograph of Prumnopitys ferruginea showing young developing root nodule inside degenerating old root nodule.
the epidermis. Stained longitudinal sections show the first two or three rows of cells immediately adjacent to the epidermis to be mainly free of hyphae and other fungal structures. Hyphae can be seen in these cells only directly beneath an entry point below the epidermis. Subsequently hyphae migrate laterally from cell to cell and radially towards the central cortex of the nodule. The cells nearest to the epidermis contain long trunk hyphae and hyphal coils. Arbuscule formation is most profuse in the central cortical cells. These cells appear packed with fungal structures including new and degenerating arbuscules and to contain little cytoplasm. Many of the cells adjacent to the endodermis are packed with degenerating fungal structures (Fig. 6d).

Within the cortex colonisation continues to be intracellular. Electron micrographs show hyphae crossing cell walls into adjacent cells, often through the thickest part of the wall (Fig. 6c).

The fungal hyphae showed morphological features typical of those described for zygomycetous fungi. Figs 5 and 6 illustrate the general appearance of fungal hyphae in the cortical cells of a nodule. The fungal profiles range from large hyphae of variable diameter to smaller more uniform hyphae. Many
of the hyphae are thin-walled, but a few have thick multi-layered walls. No septa were seen on any of the hyphae in transmission electron micrographs. The hyphae typically show thickenings and abrupt bends. The cytoplasm of living hyphae contains many small vacuoles and lipid droplets. The hyphae exhibited morphological variation in size, shape and wall thickness both in different nodules of the same species of tree, and in the different tree species studied. Whether these variations in morphology reflect different stages of development of the hyphae, different strains of fungi, or are related to the species and developmental stage of the host plant, requires further investigation and comparison with nodules containing single known strains of fungi.

In the central cortical cells hyphae are frequently collapsed and degenerated in what may be interpreted as digestive stages. In older nodules many of the cortical cells lack either hyphae or cell protoplasm.

PCR amplification of fungal DNA extracted from mycorrhizal nodules

Fifteen root nodules out of the 20 extracted produced at least one partial Glomalean rDNA sequence or a Glomalean-like restriction fragment length polymorphism. PCR and DNA sequencing results are summarized in Table 1. Some root fragments failed to amplify (data not shown). Only
two nodules produced a nonGlomalean sequence, and one of those also produced Glomalean sequences. The closest sequence relatives of the nonGlomalean fungi are taxa not known to form mycorrhizas (Hambleton et al., 1998; Okane et al., 2001; Stchigel et al., 2001). Lack of amplification may be due to: decayed nodule contents; DNA shearing during sonication which was used to clean outer nodule surface; mispriming; and/or polymerase inhibition by root compounds. Four root nodules from three Podocarpaceae species produced two types of sequences: one within the *Glomus intraradices/mosseae* clade, and one within the *Archaeospora* clade (Fig. 7). *Glomus*-like sequences were detected in all four Podocarpaceae species and *Archaeporospora*-like sequences were absent only from *Dacrydium cupressinum*. All sequences were

Fig. 5 The early stages of colonisation of root nodules by fungal hyphae. (a) and (b) hyphae in exodermis of *Prumnopitys taxifolia* nodule. Hyphae are intracellular and burrow straight through the exodermis into the adjacent cortical cells. The presence of tannins does not deter fungal entry into the exodermis (d). (c) cortical cell of *Dacrydium dacrydioides* nodule as yet uncolonised by fungal hyphae. The cell is highly vacuolated with a layer of cytoplasm around the edge. A large nucleus and amyloplasts full of starch lie adjacent to the cell wall.
Fig. 6 Transmission electron micrographs of later stages in fungal colonisation of mycorrhizal root nodule. (a) and (b) trunk hyphae with arbuscules surrounded by host cytoplasm (arrows). N = host nucleus. HV = host vacuole. (c) and (d) cells of central nodule packed with fungal debris and degenerating arbuscules.
Table 1 Podocarpaceae root nodule fungi. Five root nodules from four species (P.f., Prumnopitys ferruginea; D.d., Dacrycarpus dacrydiodes; D.c., Dacrydium cupressinum; P.t., Prumnopitys taxifolia) were tested for amplification with 10 fungal-specific primer sets in one-step or two-step (nested) polymerase chain reactions. Amplified products were screened by restriction fragment length polymorphisms and all unique products were sequenced (bold). Sequence matches to GenBank accessions with > 90% identity have been putatively assigned to *Glomus* (Glom.), or *Archaeospora* (Arch.) with the nearest taxon and sequence identity value indicated between parentheses.

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<th>Nodule</th>
<th>ITS1F/ITS4</th>
<th>NS1/NS8</th>
<th>GLOM1070R/NS1</th>
<th>LECT1670/ITS4</th>
<th>ACAU1660/ITS4</th>
<th>GLOM1310/ITS4</th>
<th>N5S/ITS4</th>
<th>GIGA5.8R</th>
<th>ARCH1311/ITS4</th>
<th>N5S/11/ITS4</th>
<th>NS1/ITS4</th>
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<td>P.f. 5</td>
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<td>D.d. 6</td>
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<td>D.d. 8</td>
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P. t. 16 – – – – – Glom. – –

Glom. (G. proliferum 96%)

P. t. 17

Ascomycete (Antarctomyces 97%)

––– – – – – Arch.

(A. trappei 96%)

Glom. (G. mosseae 95%)

P. t. 18 – – – – –

Glom.

(G. intraradices 96%)

–

Arch.

(A. trappei 96%)

–

Arch.

(A. leptoticha 97%)

P. t. 19 – – – – – – – – – –

P. t. 20 – – – – – – – – – –

Nodule ITS1F/ITS4

NS1/GLOM1070R

GLOM1310/NS8

NS5/ITS4

LECT1670/ITS4

NS5/ITS4

ACAU1660/ITS4

NS5/ITS4

GLOM1310/ITS4

NS5/ITS4

NS5/GIGA5.8R

NS5/ITS4

ARCH1311/ITS4

NS1/ITS4

NS11/GLO1375R

NS1/ITS4

NS1/ARC1375R

Table 1

continued

manually aligned to the alignment by Redecker et al. (2000). The five longest sequences (999–1709 bp) were used for the phylogenetic analysis presented in Fig. 7. These five sequences represent all sequence types retrieved in this study. The DNA sequences generated have been deposited in GenBank under accession numbers AF452624-AF452636.

Discussion

Our observations support the experimental evidence of earlier reports that members of the Podocarpaceae form nodules on their roots as a constitutive feature of their development, and these nodules are subsequently colonised by arbuscular mycorrhizal fungi. Podocarp cuttings produced nodules after 4 months in sterile soil with no trace of fungi present in the root or nodule cortex, confirming earlier experiments by Baylis et al. (1963) and Kahn (1967). Also we never observed fungi in the very young pale-coloured nodules of roots collected in the field. Further evidence of root nodules developing prior to fungal colonisation comes from a study of a species of the related Araucariaceae. Developing nodules in seedlings of *Wollemia nobilis* (Araucariaceae) both transplanted from the field, and grown from seed, contained no fungal colonisation, while mature nodules collected from the field were heavily colonised (McGee et al., 1999).

This situation appears to contrast with that in the only other mycorrhizal root nodules described to date. In the angiosperm family Casuarinaceae, Duhoux et al. (2001) found that nodules formed only on seedlings grown in nonsterile soil, after the colonisation of the root cortical tissue.

For the podocarps it is unlikely that the nodules are lateral roots whose developmental pattern has been modified by the endosymbiont. Rather the developmental pattern appears to be already determined by the host-plant. As Kahn (1967) describes, the nodule develops its distinctive cell pattern before emergence, so very early on diverges from the developmental pathway of a lateral root.

This raises interesting questions about the mode of infection of nodules. As the analysis of the young white nodules shows them to be free of fungal hyphae, it is unlikely that any infection of the nodules occurs from the root cortex by means of intraradical hyphae. The situation would appear to be the same as in angiosperms where infection does not spread internally from parent to branch roots, but every new rootlet is re-invaded from the soil. In fact the progression of fungal infection of a nodule is illustrated in Figs 5 and 6. The presence of tannins in the epidermal cells does not appear to act as a deterrent to intracellular invasion by fungal hyphae. The epidermis forms a homomorphic layer, in contrast to the dimorphic exodermis of several angiosperm mycorrhizal roots where the presence of suberin in the long cells deters fungal entry and the fungus enters the root through short ‘passage’ cells which remain unsuberised (Brundrett & Kendrick, 1990).

As the nodules are quite soft when they first appear, it is
possible that invasion by infection units occurs before the epidermal cells thicken. Fungal hyphae can be visualised on the surface of the nodules, although only a few appressoria have been observed. In the case of *Prumnopitys ferruginea*, where the young nodule is frequently found developing from the same part of the pericycle as the older disintegrating nodule (Fig. 2d), fungal hyphae can be seen extending from the cortex of the old nodule to the epidermis of the new. It is possible in the case of this species that infection may occur directly from the old nodule to the newly developing nodule.

As the nodules do not elongate in the manner of lateral roots, each nodule remains a discrete fungal colony. The absence of fungal spread from mycorrhizal nodules is atypical of mycorrhizal colonies in roots generally, where the hyphae continue to spread in the root system as it elongates. However the fungal colony in the long roots of kahikatea seedlings transplanted from natural to sterile soil continued to develop as a typical mycorrhiza. The podocarp root system would therefore appear to maintain two distinct types of fungal colony, and raises interesting questions about the role and development of each type. Experiments with single strains of fungi may provide insight into the ability of a single species to colonise both roots and nodules, and on the contrasting modes of infection and development of the mycorrhizas.

![Fig. 7 Phylogenetic placement of arbuscular mycorrhizal fungi from nodules of *Prumnopitys taxifolia*, *P. ferruginea*, *Dacrycarpus dacrydioides* and *Dacrydium cupressinum* (bold) using a previously published 18s rDNA dataset (Redecker et al., 2000). This topology is the result of neighbor-joining. Partial sequences are indicated by italicised taxon labels. Bootstrap values > 70% are near corresponding branches and branches present in the strict parsimony consensus of 15 trees are highlighted.](image)

cortex, which is an impermanent tissue with a limited lifespan. Older parts of the root are not invaded after the primary cortex has sloughed off. Whatever their physiological role, there may be some basis to the idea that the nodules, on which the cortex is annually renewed, are an adaptation for retaining the fungal symbiont after the parent roots have shed their cortex (Baylis et al., 1963). How long individual nodules remain functional as mycorrhizal units remains to be determined.

In New Zealand, the mixed podocarp-broadleaved forests grow on soils derived from glacial outwashs which are extremely low in phosphorus. The formation of mycorrhizal nodules is likely to have considerable ecological significance for growth in these habitats. An early study by Morrison & English (1967) showed that AM infection stimulated phosphate uptake by mycorrhizal nodules of Agathis australis. Other studies (Bergersen & Costin, 1964; Becking, 1966) found that mycorrhizal nodules were able to fix small but significant quantities of atmospheric nitrogen, although this finding was disputed by Furman (1970) and Baylis (1969). However, arbuscular mycorrhizas may play more of a role in nitrogen uptake than previously thought. An arbuscular mycorrhizal fungus has been shown to both enhance decomposition of and increase nitrogen capture from complex organic material in soil (Hodge et al., 2001). If this is true in nature, in the highly organic soils of the rainforest, this ability may have a particular relevance.

However, the nature of the AM association, and the role of the mycorrhizal nodules requires further study. While the fungal colonisation of the nodules represents a typical AM association in the uptake fungus. The role of the mycorrhizal nodules and whether they act in the manner of a typical AM association in the uptake and translocation of nutrients remains to be determined.

Sequence data from the 18s rDNA of the ribosomal genes revealed that several lineages of glomalean fungi colonised the nodules. Indeed the root nodules were small communities of AM fungi. Three of the podocarps, Prumnopitys ferruginea, P. taxifolia and Dacrycarpus dacrydiodes, contained AM fungi belonging to the newly characterised family Archaeosporaceae, an ancestral clade basal to existing Glomalean families (Morton & Redecker, 2001). One nodule of Dacrycarpus sampled contained two lineages of Archaeospora. In fact, we found different lineages of fungi in root nodules of Dacrydium and Prumnopitys growing in the same area of the Okarito forest and a member of the Glomus mosseae clade.

This first molecular study of the species of Glomales present in root nodules has produced a list of species new to New Zealand. In a survey of New Zealand Endogonaceae Hall (1977) identified spores in the South Island’s east coast mixed podocarp forests. The only species we found in common is Glomus mosseae. It is possible that the east and west coasts of New Zealand’s South Island have different populations of Glomalean fungi. Or our results bear out the view that spores found in the soil are not necessarily the species colonising the trees. Hall (1977) found that there were few spores in the bush soils in New Zealand, and put this down to the high density of roots in the soil, encouraging the ready spread of mycorrhizal infection from root to root. This illustrates the importance of molecular taxonomy in identifying species of fungi in mixed communities. It is likely that some of the taxa sampled are new to science as the internal transcribed spacers shared no detectable similarity to accessions currently available in GenBank (data not shown).

In this article aspects of the structure of podocarp mycorrhizal nodules have been described, with reference to four species of Podocarpaceae in New Zealand. This study has established that individual nodules are colonised by fungal hyphae entering epidermal cells from the surrounding environment, and that each nodule may be a small colony containing several different lineages of glomalean fungi. However, their function as typical mycorrhizas has yet to be established. There is an indication from this study of a degree of specificity in the lineages of fungi colonising the different tree species. Further research is required to explore the degree to which individual species of tree may preferentially form associations with different species of glomalean fungi.

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References


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