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Podocarp Roots, Mycorrhizas, and Nodules

Ian A. Dickie and Robert J. Holdaway

ABSTRACT. Podocarps are often found on low-nutrient soils and further exacerbate nutrient limitation through the production of recalcitrant litter. Understanding podocarp ecology therefore depends on understanding the root adaptations of podocarps to obtain nutrients. The roots of podocarps are heterorhizic (having two root forms) with abundant spherical determinate short roots, a unique form of largely unknown function, as well as indeterminate fine roots. There is no strong evidence for nitrogen fixation in the nodules. Although nodules are frequently and abundantly colonized by arbuscular mycorrhizal fungi, they are neither required for mycorrhizal infection nor dependent on mycorrhizal infection for their development. The fungal symbiont community associated with podocarps does not appear to be distinctive from that of other plants at a global scale, although it may differ from co-occurring plants within a site. Perhaps the most obvious, yet largely overlooked, hypothesis is that the form of the nodules is the function. The presence of nodules permits a doubling of root cortex volume, while simple geometry indicates that a spherical form involves the lowest possible cost in producing cell wall and membrane material per unit of root volume. Increased root volume permits greater arbuscular mycorrhizal interaction, and hence, nodules may be analogous to the determinate ectomycorrhizal roots of the Pinaceae as a structure to maximize fungal interactions at minimal root construction cost.

INTRODUCTION

Podocarps (gymnosperms in the Podocarpaceae) grow on a range of soils from relatively fertile (e.g., C:N and C:P ratios of 12.6 and 394; Solomon et al., 2002) to infertile soils with most mineral nutrients locked into organic forms (e.g., C:N ratios = ~40, C:P ratios > 1,000; Armesto et al., 1995; Coomes et al., 2005). Nonetheless, it is the ability of podocarps to grow and compete on infertile sites that explains their occurrence in lowland tropical habitats (e.g., Kitayama et al., this volume) and their dominance on older, relatively phosphorus-limited temperate sites (Richardson et al., 2004; Coomes et al., 2005; Coomes and Bellingham, this volume). On higher-phosphorus soils, podocarps are more likely

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to be outcompeted by ferns and angiosperms (Coomes et al., 2005; Carswell et al., 2007) and may show negative growth and survival responses to phosphorus or mixed-fertilizer addition (Carswell et al., 2003; Pareliussen et al., 2006). Where present, podocarps can exacerbate mineral nutrient limitation through the production of relatively low quality, high-carbon leaf litter, which can lock mineral nutrients into unavailable forms (Wardle et al., 2008). This litter decomposes so slowly that it can remain obvious more than 40 years after the death of a podocarp tree (Wardle et al., 2008). Understanding podocarp ecology therefore requires an understanding of the mineral nutrient uptake strategies that allow the persistence of podocarps on low nutrient soils.

In this chapter we examine the root adaptations of podocarps that permit efficient mineral nutrient acquisition from low-nutrient soils, both in terms of maximizing nutrient uptake and minimizing costs. We focus on two key questions: (1) Are there unique aspects of the mycorrhizal associations of podocarps that permit them greater mineral nutrient access on low-nutrient soils? (2) What, if any, is the functional role of nodules in podocarp mineral nutrient uptake?

MYCORRHIZAL ASSOCIATIONS OF PODOCARPS

Podocarps are arbuscular mycorrhizal, forming symbiotic associations between plant roots and fungi in the Glomeromycota. In these symbioses the plant provides carbon to the fungus, and the fungus greatly increases plant acquisition of phosphorus (Smith and Read, 2008). The presence of arbuscular mycorrhizas is typical of more than 50% of gymnosperm species, with the remainder, comprising the Pinaceae and Gnetaceae, primarily forming ectomycorrhizas (Brundrett, 2009). This potentially reflects a close phylogenetic relationship between these two families (Hajibabaei et al., 2006; Wu et al., 2007), although Brundrett (2009) argues for separate evolution of ectomycorrhizas in these groups. Although there are sporadic reports of ectomycorrhiza in other gymnosperms (e.g., Cupressaceae, *Wollemia*), these appear to reflect misdiagnosis or atypical definitions of ectomycorrhizas (Brundrett, 2009). Arbuscular mycorrhizas are also typical of 74% of angiosperms, including many of the plant species frequently associated with podocarps (Brundrett, 2009).

Arbuscular mycorrhizal infection of podocarp roots is generally extremely dense. In New Zealand temperate podocarps, there are reported infection levels of 88%–96% of root length (Hurst et al., 2002); our own observations indicate that nearly 100% of available root length (fine roots capable of forming arbuscular mycorrhizas) is infected across a number of temperate podocarp species (I. A. Dickie, unpublished data). These levels of arbuscular mycorrhizal infection are higher than many other plants and significantly higher than some, but not all, co-occurring angiosperms.

Arbuscular mycorrhizas benefit plants by increasing the effectiveness (extent of soil exploration) and efficiency (benefit:cost ratio) of phosphorus uptake (Koide et al., 1999). Phosphorus is relatively immobile in soil, so the extensive hyphae of arbuscular mycorrhizal fungi provide a greatly increased ability to explore the soil volume. The relative dependence of plants on mycorrhizal infection is generally strongly correlated with plant root traits (Baylis, 1975; St John, 1980). Species with high specific root length (length/mass) and abundant and long root hairs are expected to have relatively lower dependence on mycorrhizal fungi than species with thick, higher-cost roots and fewer root hairs.

There have been few studies of podocarp root morphology, but our own observations suggest a moderately coarse root system (mean diameters ~0.60 mm; specific root length 711 ± 85 cm g^{-1}, mean ± standard error), with abundant root hairs in some species (Figure 11.1). Observed root diameters are consistent with the concept of podocarps having a high mycorrhizal dependency (St John, 1980); however, the production of abundant root hairs in some podocarps is not. The presence of abundant and long root hairs and mycorrhizas may indicate they have different functions in mineral nutrient uptake or that they have complementary functions under conditions of poor mineral availability.

IDENTITY OF THE FUNGAL SYMBIONTS OF PODOCARPS

Like all arbuscular-mycorrhizal symbiosis, the fungi that form arbuscular mycorrhizas with podocarps are in the Glomeromycota (Schüßler et al., 2001). The fungi appear typical of arbuscular mycorrhizal communities in general, with a dominance of *Glomus* species and less common *Archaeospora* and Diversisporaceae (Russell et al., 2002; Wubet et al., 2006). Two studies might be taken to suggest a degree of host specificity to podocarps. Wubet et al. (2006) found distinct fungal species associated with co-occurring *Juniperus procera* (Cupressaceae) and *Afrocarpus falcatus* (Podocarpaceae). Further, in a study of four species of podocarps, Russell et al. (2002) found little similarity between sequences of Glomeromycota

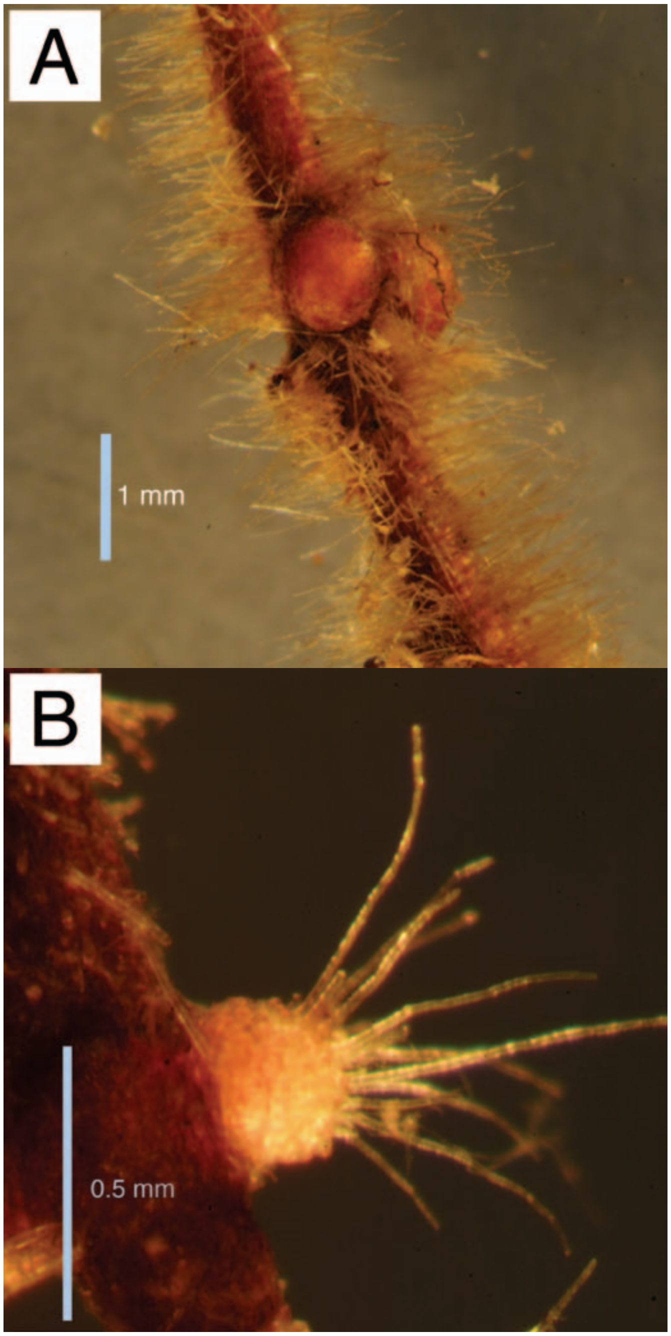


FIGURE 11.1. Abundant root hairs on podocarps occur both (A) on long roots, as seen on *Prumnopitys ferruginea*, and (B) sometimes on nodules, as seen on *Phyllocladus alpinus*. Images by the authors.

from podocarp roots and existing sequences in GenBank (http://www.ncbi.nlm.nih.gov). Nonetheless, at least the results from Russell et al. (2002) may reflect the paucity of Southern Hemisphere Glomeromycota sequences (particularly in 2002), rather than true host specificity. Using the ribosomal (18s, internal transcribed spacer [ITS]) sequence data from Russell and colleagues (GenBank Accession numbers AF452624–AF452636), we reexamined these sequences against GenBank to assess affinities with more recent collections (Table 11.1). Twelve of the 13 sequences matched at 95% or greater maximum identity to other sequences in GenBank, of which three matched only sequences from New Zealand, five had nearest matches to sequences from elsewhere in the Southern Hemisphere, and four had nearest matches to sequences from the Northern Hemisphere. There was no evidence of any specificity of these fungi to podocarps—the nearest matches included liverworts, grasses, and a range of angiosperms, with no matches to other podocarps. Therefore, the arbuscular mycorrhizal fungi colonizing podocarps may be distinct from other host plants within a site (Wubet et al., 2006), but on a broader scale the fungi associating with podocarps do not appear to be particularly distinctive. Apparent host specificity within sites may reflect the strong effects of individual podocarp trees on soil chemical and biotic properties (Wardle et al., 2008) influencing local fungal community composition.

ROOT NODULES OF PODOCARPS

The roots of the podocarps are characterized by abundant spherical protuberances, often referred to as "nodules" (Figure 11.2). Thus, podocarps are heterorhizic, having two distinct root forms: long indeterminate roots and determinate nodules. Initial nodule formation typically occurs as regularly spaced spheres developing in multiple rows along the length of fine roots, with substantial variation among plants in the regularity of nodule formation. Nodules arise from the root pericycle, similar to lateral root development (Russell et al., 2002), and are characterized by the absence of any apparent meristematic tissue or root cap (Khan and Valder, 1972). As nodules age, epidermal cell walls become thicker, and the color of the nodules becomes increasingly dark (Russell et al., 2002). Russell et al. (2002) noted that starch reserves were high in nodule cortical cells prior to arbuscular mycorrhizal colonization and were depleted in heavily colonized cells. In some cases, older nodules are shed and can become abundant in soil. After shedding old nodules, nodule formation may be reinitiated at the same location. In an interesting variation, it is also possible for apical growth to resume, potentially contributing to the formation of chains of nodules (discussed below). The unique form of podocarp nodules has led to a number of hypotheses about their functional

TABLE 11.1. Re-BLAST (basic local alignment search tool) searching of sequences from Russell et al. (2002) against GenBank, showing best match (if greater than 95% similarity) to arbuscular mycorrhizal associates of known host plants or plant communities. There is no evidence of host specificity to podocarps; N/A indicates not applicable.

Sequence from Russell et al. (2002)	Sequence name	Best match (>95%)	Identities	Host plant	Host plant taxonomic group	Collection location
AF452624	PODO17.1	EU417643.1	509/519 (98%)	*Sciaphila ledermannii*	Triuridaceae	Cameroon
AF452625	PODO17.2	AF452625.1	305/305 (100%)	*Juglans neotropica*	Juglandaceae	Ecuador
AF452626	PODO17.3	AJ716326.1	878/886 (99%)	*Marchantia foliacea*	Marchantiales	New Zealand
AF452627	PODO16.1	AJ699064.1	552/557 (99%)	*Marchantia foliacea*	Marchantiales	New Zealand
AF452628	PODO16.2	AJ699068.1	1,010/1,046 (96%)	*Marchantia foliacea*	Marchantiales	New Zealand
AF452629	PODO1.1	DQ336488	173/181 (95%)	*Guarea pterorhachis*	Meliaceae	Ecuador
AF452630	PODO1.2	EU152178	508/510 (99%)	*Tabebuia chrysantha*	Bignoniaceae	Ecuador
AF452631	PODO1.3	AM384971	373/384 (97%)	*Thymus pulegioides*	Lamiaceae	Switzerland
AF452632	PODO7.1	AM268195	549/564 (97%)	*Grassland*	Various	Finland
AF452633	PODO7.2	FJ483156	520/529 (98%)	*Freshwater marsh*	Various	United States
AF452634	PODO7.3	AM420382	566/580 (97%)	*Aquatic macrophyte*	Unclear	Norway
AF452635	PODO18.1	EU159171	499/499 (100%)	*Neotropical rainforest*	Various	Ecuador
AF452636	PODO18.2	None	Best match only 94%	N/A	N/A	N/A

significance. Despite nearly 100 years of research on this subject, a clear explanation has yet to emerge.

Plant Phylogeny and Occurrence of Nodules

Within the gymnosperms, nodulated roots are present in the Podocarpaceae, Phyllocladaceae, and Araucariaceae, which form a single phylogenetic group, and *Sciadopitys* (Khan and Valder, 1972), which Stevens (2001–2009) suggested is a distinct but closely related clade (Figure 11.3). Nodules in the Araucariaceae and *Sciadopitys* were reported as less regular and more elongated and variable in size than nodules in podocarps (Khan and Valder, 1972), but we have observed regular, spherical nodules in *Agathis australis* (Araucariaceae; personal observation). Nodules are absent from the Cupressaceae and Taxaceae.

Outside of the gymnosperms, mycorrhizal nodules have been reported in a number of angiosperms, including *Gymnostoma* (Casuarinaceae; Duhoux et al., 2001), *Aesculus* (Sapindaceae; Khan, 1972), and several Caesalpinaceae (Béreau and Garbaye, 1994). It is not clear that these other nodules are physiologically or genetically related to podocarp nodules. Khan and Valder (1972) outlined two traits as defining true podocarp-type nodules: (1) development as a normal feature of roots, not being dependent on any microorganism for their development (Baylis, 1969), and (2) being fully differentiated structures with no root cap or apical meristem and with an endodermis surrounding and overarching the vascular strand.

The second of these defining traits must be taken as temporally dynamic, as both the initial growth and, particularly, the reinitiation of apical growth in some nodules implies an apical meristem. Nonetheless, none of the nonpodocarp nodulated roots have been shown to fit these criteria. In *Gymnostoma*, nodules do not form as a normal feature of the root, as the presence of soil microorganisms is required to induce nodule formation. Additionally, nodules in *Gymnostoma* have an apical meristem, and no evidence of an endodermis overarching the vascular strand was reported (Duhoux et al., 2001). The nature of nodule formation in *Aesculus* remains unclear, because the one attempt to synthesize nodules in the absence of soil microbes failed to maintain soil sterility over the course of the experiment (Khan, 1972).

There are occasional reports of nodules in other groups; however, these appear to be based on confusion between beaded roots and true podocarp-type nodules (Khan and Valder, 1972). Beaded roots are a commonly

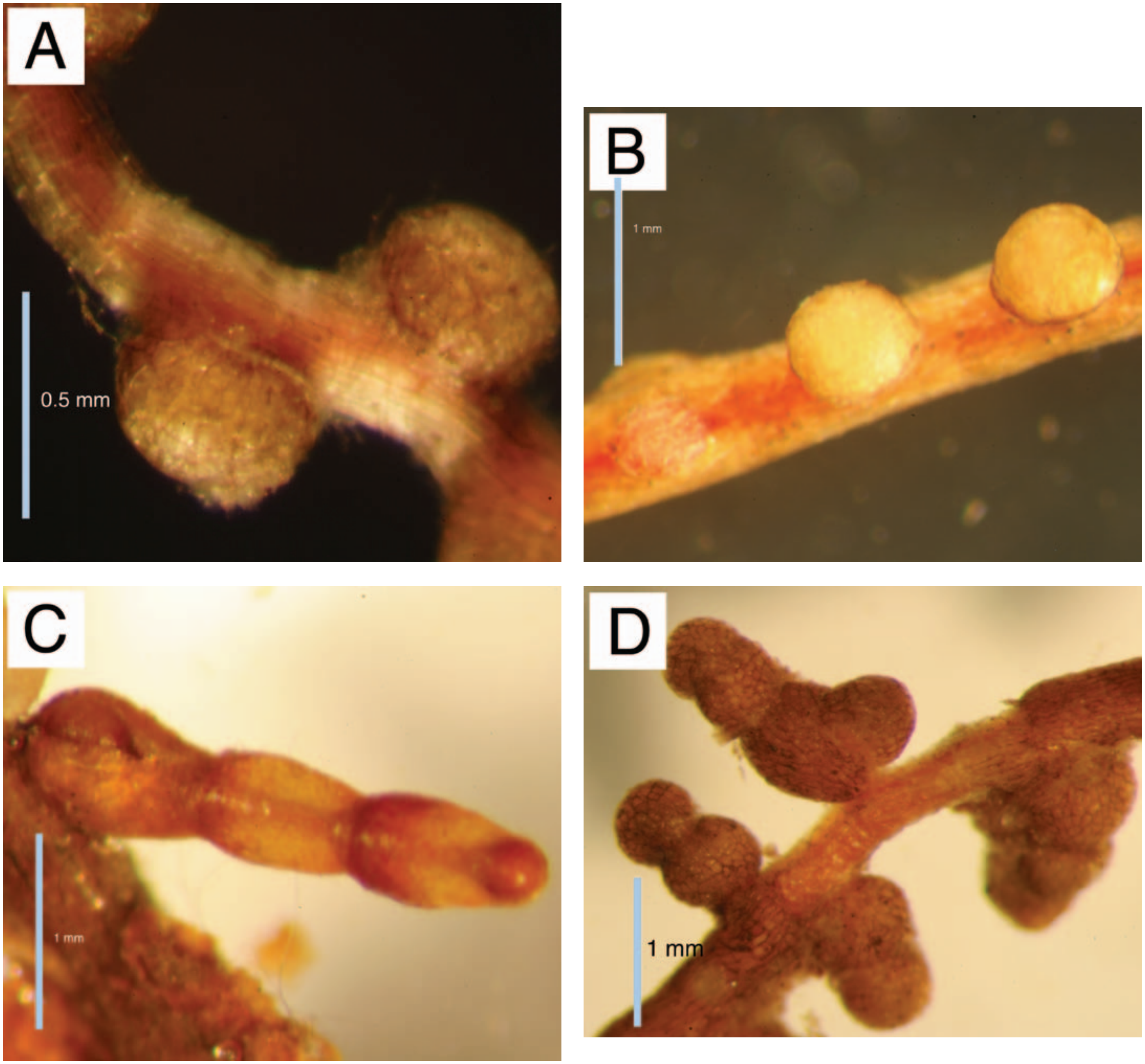


FIGURE 11.2. Examples of variation in form among nodules and roots: (A) very small nodules on *Lepidothamnus intermedius*, (B) formation of nodules (three stages of development) of regularly spaced uniform nodules in *Dacrydium cupressinum*, (C) beaded roots (*not* nodule) in *Dacrydium cupressinum* (note the stele passing through beads and to the end of root with no overarching cortical cells), and (D) chained or beaded nodules in *Manoao colensoi*. A range of species are shown, but significant variation can also be found within species. Images by the authors.

observed root form in many tree species, where roots are regularly segmented by narrow constricted regions. Although beaded roots have occasionally been reported as being either ectomycorrhizal (Mineo and Majumdar, 1996) or arbuscular mycorrhizal structures (Grand, 1969), it appears that beaded roots are not directly related to mycorrhizas or other soil organisms (Kessler, 1966; Beslow et al., 1970; Thomson et al., 1990). The development of beaded roots may reflect episodic growth linked to fluctuating soil moisture (Kessler, 1966), although this result

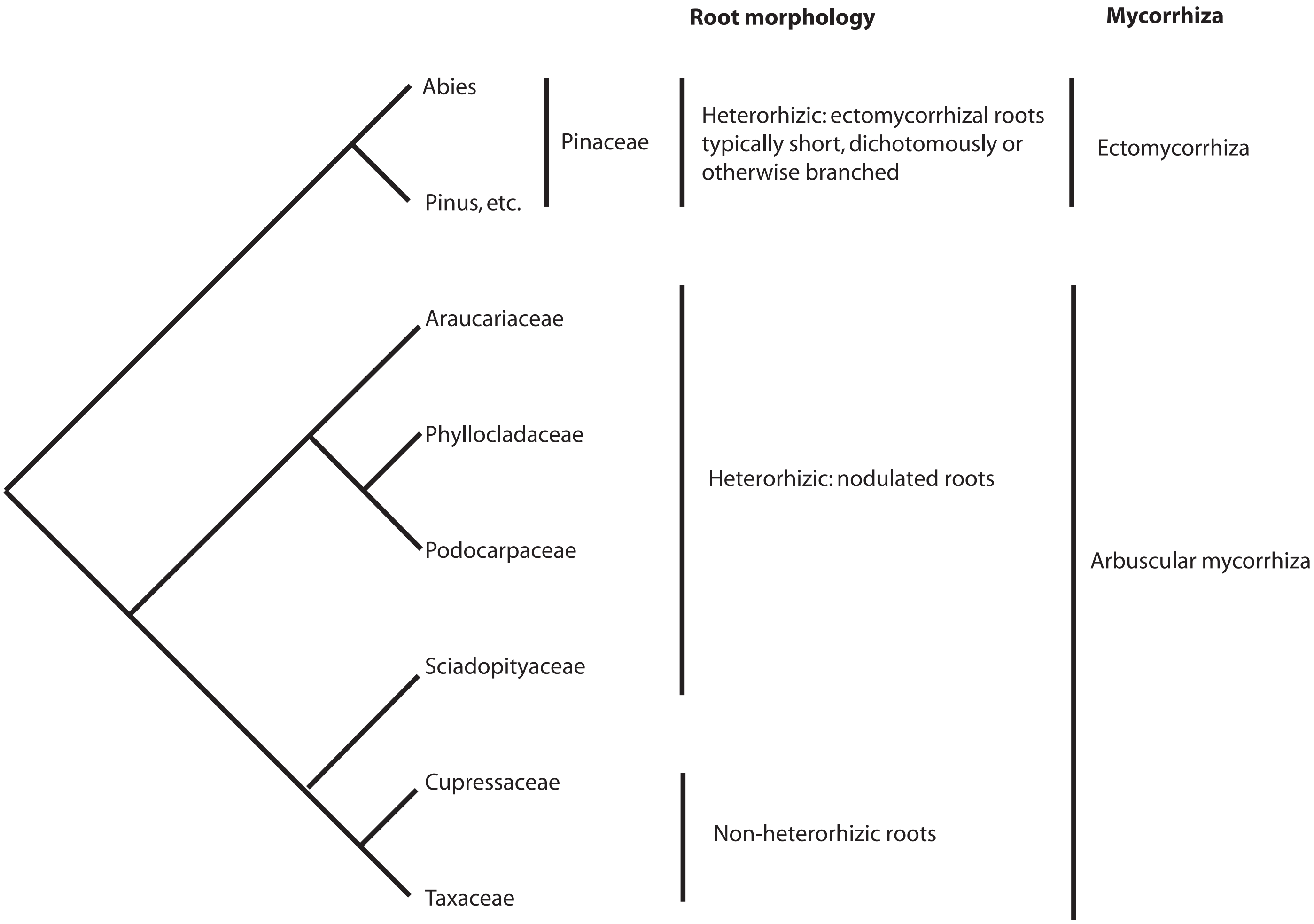


FIGURE 11.3. Phylogeny of the Pinales with mycorrhizal status and notes on root nodule morphology. Phylogeny follows Stevens (2008), who attempted to reflect current consensus. The podocarps share arbuscular mycorrhizas with the Cupressaceae and Taxaceae, but the presence of heterorhizic roots (i.e., short determinate roots distinct from long roots) shows similarity to the Pinaceae. Note that arbuscular mycorrhizas are also present in the Pinaceae, but ectomycorrhizas dominate (Horton et al., 1998).

is not consistent across studies (e.g., Beslow et al., 1970). Nonetheless, beaded roots retain meristematic tissue and have no overarching cortex and are, therefore, distinct from true podocarp-type nodules. Some podocarps form beaded roots in addition to nodules (Figure 11.2c), and some podocarp nodules develop apically on previously formed nodules, creating a beaded-nodule chain (Figure 11.2d). Perhaps it is not surprising that this has led to considerable confusion.

Nitrogen Fixation and Podocarp Nodules

There have been a number of studies that claim to show low levels of nitrogen fixation in podocarp nodules (e.g., Morrison and English, 1967; Grobbelaar et al., 1971) and others that fail to find any nitrogen fixation (Baylis, 1969). Morrison and English (1967) reported the presence of an endophyte resembling an actinomycete but give no further details. There was also a recent report of the isolation of *Rhizobium* (along with detection of weak nitrogenase activity) from *Podocarpus macrophyllus* roots with nodules (Huang et al., 2007). Nonetheless, on the whole, the evidence for nitrogen fixation in nodules is weak. The levels of nitrogen fixation reported in podocarp nodules are typically described as very low. Where low levels of nitrogen fixation have been reported, they may reflect nitrogen fixation by free-living rhizobium in soils or associated with roots, rather than a true symbiosis.

This was effectively demonstrated by Silvester and Bennett (1973), who showed that acetylene reduction (a proxy for nitrogen fixation) was uniformly associated with the soil or surface of roots and nodules of five species of nodulated gymnosperm, with no evidence of acetylene reduction in root nodules following a relatively mild surface sterilization.

The interest in nitrogen fixation in podocarp nodules appears to largely be driven by a superficial resemblance to rhizobial nodules, leading Khan and Valder (1972:40) to state "It is unfortunate that the word 'nodule' should have been applied at all…Had they been given a different name to begin with, it is doubtful whether confusion would ever have arisen in the literature concerning their function and mode of origin." Confusion between mycorrhizas and nitrogen fixation is by no means restricted to podocarps; some of the history and causes of this confusion are reviewed by Mikola (1986).

Although there is no good evidence of symbiotic nitrogen fixation in podocarp nodules, the potential importance of asymbiotic free-living or root-associated nitrogen fixation should be an area for further investigation. Asymbiotic nitrogen fixation can supply significant amounts of nitrogen in *Agathis australis* forests (Silvester, 2000) and in other forest systems (Reed et al., 2008) and may play a similar role in podocarp forests (Silvester and Bennett, 1973). Alternatively, a lack of available phosphorus under podocarps, partially driven by low litter quality, may limit the extent of asymbiotic fixation (Reed et al., 2008).

Arbuscular Mycorrhizal Infection of Nodules

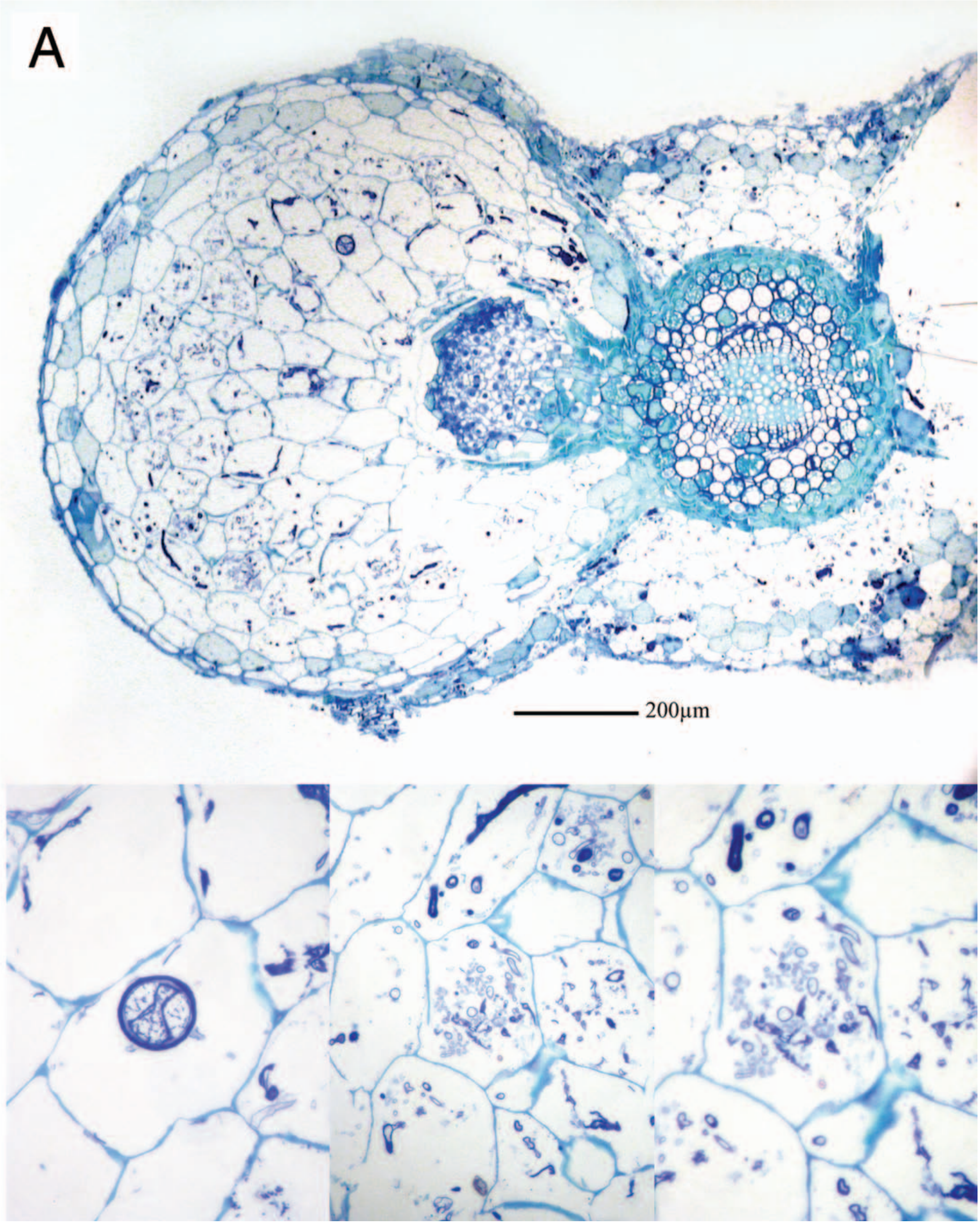
The roots and nodules of podocarps are abundantly colonized by arbuscular mycorrhizal fungal hyphae (Figure 11.4). Nonetheless, Breuninger et al. (2000) note that the extensive arbuscular mycorrhizal structures in normal fine roots of *Araucaria* suggest that nodules may not play a major role in mycorrhizal associations. Our own observations support this, with extensive infection of long root cortical cells common in podocarps. Further, nodules do not require mycorrhizal fungi for their formation (Baylis, 1969). The presence of arbuscular mycorrhizal fungi is therefore neither a prerequisite for, nor dependent on, nodule formation.

There is some confusion around the question of whether extraradical mycorrhizal hyphae are associated with nodules. The extraradical hyphae are the portion of the arbuscular mycorrhizal fungal mycelium where active nutrient uptake from the soil solution occurs; hence, a lack of extraradical hyphae would imply an atypical, perhaps parasitic, mycorrhizal association. The outermost layers of podocarp nodules were reported as "mainly free of hyphae" by Russell et al. (2002), but McGee et al. (1999) showed sparse hyphae in epidermal cells. Epidermal cells in podocarp nodules have notable thickening of cell walls on mature nodules, which may lend support to the idea of limited fungal penetration through the epidermis. Nonetheless, the occurrence of dense fungal colonization in the cortex with only sparse colonization of outer layers is actually quite typical for all plant species: arbuscular mycorrhizal fungi penetrate a small number of epidermal cells, pass through the "exodermis" layer just below the epidermis (if present), and then proliferate in the cortex. Extensive arbuscular mycorrhizal hyphae on the surface of nodules of *Araucaria* were shown by Breuninger et al. (2000). In our own observations of *Podocarpus*, *Dacrydium*, and *Prumnopytis* we have observed frequent hyphae on the surface of nodules, what appear to be typical appresoria (cell penetration arises from appresoria), and hyphae that penetrate the surface of nodules. We therefore conclude that there is an extraradical mycelium with apparently typical attachment to the internal infection of root nodules by arbuscular mycorrhizal fungi and that prior reports of the absence of extraradical hyphae are incorrect.

Whether the arbuscular mycorrhizal fungi infecting nodules are distinct species from those infecting other roots on the same plant remains unknown. As observed by Russell et al. (2002), the determinate growth form of nodules places a constraint on fungal colony growth compared to long roots, as hyphal spread along a growing root is not possible. Russell et al. (2002) did observe, however, that fungal hyphae from the disintegrating cortex of old nodules were able to infect newly forming nodules.

Replacement of Nodules

Root nodules, similar to fine roots in other species, have a limited life span (Eissenstat and Yanai, 1997). Nodules may be shed by the plant, with regrowth of new nodules at the same location. In other cases, nodules are formed in strings of connected nodules, which may reflect apical regrowth. The replacement of nodules is unlikely to be driven by declines in local soil fertility as there is little to be gained by replacement in the same location. Instead, there may be physiological changes in the nodules that render them increasingly inefficient with age. At least three hypothetical mechanisms may be involved. First, arbuscules, the site of nutrient transfer, are short-lived structures in most plants. In podocarps, collapsed fungal arbuscules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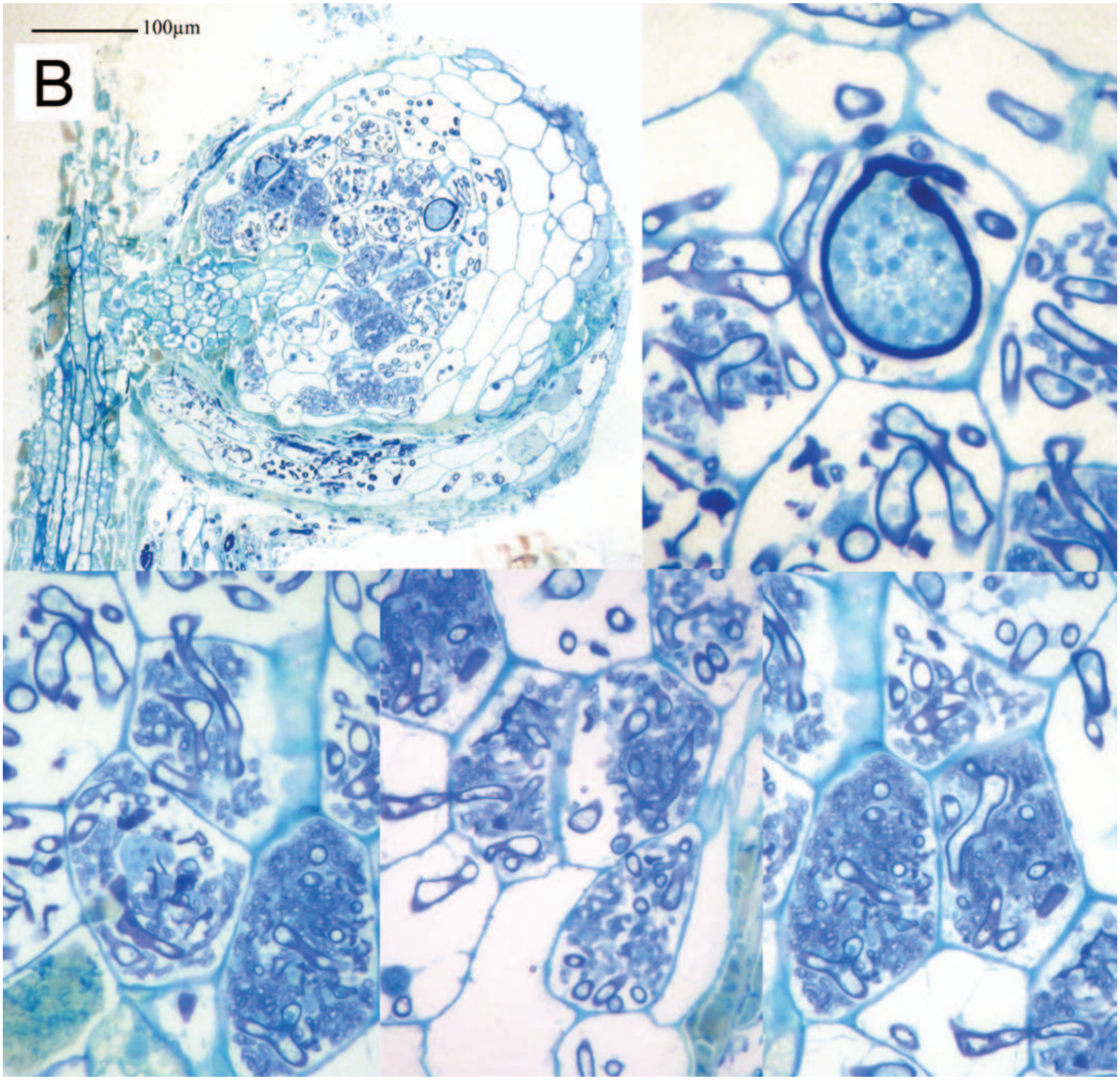

FIGURE 11.4. Internal structure of nodules from (A, *facing page*) *Podocarpus totara* and (B, *above*) *Dacrydium cupressinum* showing arbuscular mycorrhizal fungal tissue inside plant root cortical cells, with abundant arbusculate fungal coils (darker blue, fine structures within plant cells) and probable vesicles (ovoid structure in B) and spore (round structure in A). Microscopy was courtesy of Larry Peterson and Lewis Melville from material collected by the authors. Roots were stored in 2.5% glutaraldehyde in PO_4 buffer, pH 6.8, dehydrated in a graded series of ethanol to 100%, embedded in LR White resin (London Resin Company), sectioned (approximately 1–1.5 µm thickness) with glass knives, heat fixed to glass slides, stained with 0.05% toluidine blue O in 1.0% sodium borate, rinsed with water, air dried, and mounted in immersion oil under a cover glass.

have been described as abundant in some nodule root cells (Russell et al., 2002). Although fungi may, in other plants, reinfect a previously infected cell with collapsed arbuscules, this is generally rare (Smith and Read, 2008). By either shedding or apically expanding nodules, podocarps may reestablish the symbiosis, without requiring growth of extensive roots. Second, nodule starch reserves rapidly decline during arbuscular mycorrhizal fungal infection (Russell et al., 2002). The reformation of nodules may be necessary to replenish these reserves. Finally, the thickening of epidermal cell walls in mature nodules is likely to prevent infection by new fungal hyphae (combined with a decline in established infection). If there were any nonsymbiotic mineral nutrient uptake by nodules, this would also be restricted by epidermal wall thickening. Further work is needed to better understand the role of nodule senescence in podocarp mineral nutrient uptake efficiency.

Functional Significance of Nodules

The extensive mycorrhizal structures found in podocarp nodules suggest a link between these nodules and arbuscular mycorrhiza, but given that the formation of nodules is neither necessary for mycorrhizal infection nor dependent on mycorrhizal infection to occur, this link must be indirect. Perhaps the simplest hypothesis is that nodules simply permit a plant to increase its extent of arbuscular mycorrhizal infection while minimizing the cost that would be associated with developing an extensive root system (McGee et al., 1999). This hypothesis is supported by the very high levels of mycorrhizal infection often observed in podocarp roots and the frequent occurrence of podocarps on infertile soils, where extensive mycorrhizal development is likely to be beneficial.

Using *Prumnopitys ferruginea* as a model root system, we calculated the root surface area and volume associated with long roots, root hairs, and root nodules. On the basis of 30 segments of root examined, root diameters averaged 0.72 mm (±0.03 standard error); there were 88 (±16) root hairs per mm of root, with an average length of 0.78 mm (±0.02) and a root hair diameter of 0.02 mm. Average volume per nodule was 0.60 mm^3 (±0.03), and there was an average of 0.77 (±0.06) nodules per mm of root. On the basis of these measurements, the presence of root nodules permits the plant to increase total root volume by around 126% (±15%). Similar results were found for *Dacrydium cupressinum* (except that root hairs were absent), where the presence of root nodules permits the plant to increase total root volume by around 96% (±10%).

Thus, by forming nodules podocarps effectively double their root volume, permitting a much greater extent of arbuscular mycorrhizal infection than would be possible without nodules. The form of nodules is ideally suited for this purpose—a sphere creates the greatest possible increase in cortex volume at the lowest possible investment in vascular or epidermal tissue.

The economic analogy may be furthered by the observation that some podocarps shed their nodules while retaining long roots. If shedding nodules is physiologically necessary to maintaining mycorrhizal symbioses, then plants may minimize costs by forming very short roots with maximal volume. Where root hairs form on the nodule epidermis, the reformation of nodules with young epidermal cells may also permit plants to reestablish root hair growth (Figure 11.1b).

Further understanding of the mineral nutrient economy of podocarp nodules might benefit from comparison with the determinate, branching roots typical of ectomycorrhizal plants. The majority of ectomycorrhizal plants have heterorhizic roots, responding to fungal infection by branching or bifurcation of short, generally determinate roots. Although the function of this branching has not been widely evaluated, it seems logical to assume that localized root branching permits a greater volume of interaction between the plant and fungal symbionts and may be analogous to the development of nodules infected by arbuscular mycorrhizal fungi in podocarps. It would be particularly interesting to examine the formation of Y-shaped bifurcated ectomycorrhizal short roots in the Pinaceae (Preston, 1943) to determine whether this process is under the same genetic and physiological controls in the Pinaceae as nodule formation in podocarps. A part of this analysis should question why nodules and bifurcated roots are well developed in gymnosperms but not in angiosperms and whether this reflects other constraints on root morphology, including physiological limits on minimum root diameters in gymnosperms.

OTHER NUTRIENT ACQUISITION STRATEGIES IN PODOCARPS

The most unusual nutrient acquisition strategy within the podocarps occurs in *Parasitaxus usta* (Feild and Brodribb, 2005). Endemic to New Caledonia, *Parasitaxus* is parasitic on *Falcatifolium taxoides* (also Podocarpaceae) and is apparently the only parasitic gymnosperm. Water relations of *Parasitaxus* are similar to parasitic woody plants (e.g., mistletoe), but significantly enriched carbon-13 relative to plant host and the presence of abundant fungal hyphae suggest that photosynthate parasitism involves a fungal partner (Feild and Brodribb, 2005). Thus, *Parasitaxus* may be simultaneously a direct and an indirect parasite for different resources.

SYNTHESIS AND CONCLUSIONS

The importance of leaf form in the success of podocarps on infertile soils has long been recognized, yet the importance of root form (particularly nodules) has remained enigmatic. On the basis of the best available evidence we suggest that podocarps do not differ in root function from other plants, at least not in any fundamental way. Evidence for nitrogen fixation or novel fungal associations is weak at best. Instead of invoking some unique *function* of podocarp roots, we suggest that the unique *form* of podocarp roots is itself key to understanding their mineral nutrient uptake strategies. Nodules on podocarp roots greatly increase root volume available for symbiotic interaction with mycorrhizal fungi at minimal cost and therefore maximize mineral nutrient acquisition efficiency. This may be further augmented by the ability of some podocarps to shed and re-form nodules, reestablishing the mycorrhizal symbiosis at minimal root construction cost. Nonetheless, this view must be taken as a hypothesis to be tested.

Despite their root and leaf adaptations, podocarps may not be the best adapted species for the environments in which they occur. Low-nutrient soils, especially those with an accumulation of organic matter, often favor ectomycorrhizal trees (Lambers et al., 2008). Although the arbuscular mycorrhizal associations of podocarps are efficient in the uptake of inorganic phosphate, ectomycorrhizal fungi have much greater capabilities for organic nutrient capture (Lambers et al., 2008). Where present, ectomycorrhizal trees may therefore gradually displace podocarps and other arbuscular mycorrhizal vegetation (Taylor et al., 2009). This process may be contributing to the gradual displacement of podocarp forests by ectomycorrhizal *Nothofagus* in New Zealand (McGlone et al., 1996) and ectomycorrhizal *Quercus* in alpine South America (Van't Veer and Hooghiemstra, 2000). Podocarps have a significant advantage over ectomycorrhizal vegetation, however, as they share arbuscular mycorrhizal fungi with many other plant species, whereas the spread of ectomycorrhizal trees may be slowed by a lack of compatible mycorrhizal symbionts (Baylis, 1980; Dickie and Reich, 2005).

Progress on understanding the mineral nutrient acquisition of podocarps has been slow, partially because of an apparent fixation (pun intended) on nodules and the possibility of nitrogen fixation. We suggest that further research would be aided by (1) an economic perspective on carbon costs and mineral nutrient efficiency of root and nodule formation in the podocarps, including any physiological constraints limiting alternative strategies such as increased specific root length, (2) an investigation into the genetic basis for nodule formation, testing whether this trait is homologous with ectomycorrhizal short roots in the Pinaceae, and (3) understanding the co-occurrence and replacement of podocarps by ectomycorrhizal trees and the implications of this for ecosystem processes.

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