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Mycorrhizae Are Present in Cycad Roots

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I. Abstract

We describe the occurrence of arbuscular mycorrhizae in the roots of *Zamia pumila* and *Dioon edule*. Seedlings were grown on native, unsterilized soil taken from local pinelands of south Florida, where *Z. pumila* occurs naturally. Arbuscules, hyphae, hyphal coils, and vesicles occur in the parenchyma cells of the root cortex, especially the half of the cortex next to the stele. Hyphae of the arbuscular mycorrhizal fungi (AMF) occur mainly in longitudinal intercellular spaces and conform to the *Acorus* type. The finest, ultimate roots have AMF, but these roots are extremely brittle, detach with the slightest disturbance, and are usually lost when plants are uprooted from the ground. No AMF were found in the cortex of coralloid roots.

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Table I
Reported occurrence of arbuscular mycorrhizae in cycads

Taxon	Reference
Ceratozamia mexicana	Vovides, 1991
Dioon edule	Vovides, 1991; present report
Lepidozamia hopei	Reddell et al., 1996
Macrozamia reidlei	Brundrett & Abbott, 1991
Macrozamia communis	Brockhoff & Allaway, 1989
Zamia pumila	Present report

Vovides (1991) previously reported that AMF occur on *Dioon edule* and *Ceratozamia mexicana,* and we reconfirm this in *D. edule*. In this species, AMF appear to be mostly associated with the outer and to a lesser extent the inner cortex. However, roots of a potted plant of *C. hildae* growing in native soil lacked AMF. When grown on low phosphorus soils, legumes are known to require AMF in order for their *Rhizobium* nodules to fix nitrogen. Without AMF, the legumes are deficient in phosphorus, which inhibits nodule production and nitrogen fixation. It is probable that cycads, with their nitrogen-fixing coralloid roots containing *Nostoc,* may also require AMF for successful nitrogen fixation when phosphorus is limiting.

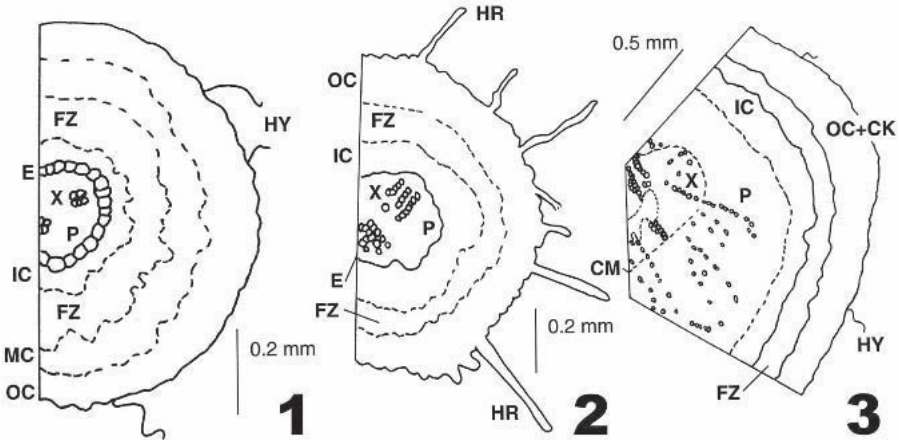
II. Introduction

The symbiotic relationship between cycad roots and the nitrogen-fixing cyanobacterium *Nostoc* is well recognized (Lindblad & Bergman, 1989; Norstog & Nicholls, 1997). However, recent reviews have not noted that mycorrhizae, root-fungal symbioses, also occur in cycads (Norstog & Nicholls, 1997; Smith & Read, 1997), even though arbuscular mycorrhizae were first reported by Vovides (1991), Brockhoff and Allaway (1989), Brundrett and Abbott (1991), and, later, Reddell et al. (1996). Here, we expand on the description of fungal colonization in two cycad genera that have arbuscular mycorrhizae and note the significance of its association with a nitrogen-fixing symbiosis. The occurrence of arbuscular mycorrhizae in cycads is summarized in Table I.

III. Materials and Methods

One-to-four-year-old seedlings of *Zamia pumila* L. were grown in Miami in pots of unsterilized native sandy soil collected in the same pine rockland habitat as wild plants. Common nearby plants included *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult., *Serenoa repens* (Bart.) Small, *Rhus copallina* L., and *Pithecellobium keyensis* Britton ex Britton & Rose. Ten-year-old seedlings of *Dioon edule* Lindl. and a three-year-old *Ceratozamia hildae* Landry & M. Wilson were grown in pots of unsterilized soil in Xalapa, Mexico. Roots were collected from unpotted plants.

Fresh or FAA-fixed roots where hand-sectioned with a razor and stained with aqueous toluidene blue for general histology. Phloroglucinol–HCl and sudan III and IV were used to test for lignin and suberin, respectively. Thick transverse sections and short segments of whole roots were cleared in 10% KOH, bleached with NH_4OH–H_2O_2, and stained with 0.05% trypan blue in acidic glycerol (Brundrett et al., 1996) for fungal observations. This material was mounted in acidic glycerine for microscopic observation and photography.



Figs. 1–3. *Zamia pumila,* transverse sections of different ultimate roots. **1.** Young region about 5 mm from tip, no secondary growth. **2.** Older region, about 10 mm from tip, root hairs happen to be in this region, beginning of secondary growth. **3.** Older root with developed secondary growth. *Key:* CK = cork; CM = cambium; E = endodermis; FZ = fungal zone; HR = root hair; HY = hyphae; IC = inner cortex; MC = middle cortex; OC = outer cortex; P = phloem; X = xylem. Scale bars indicated for each.

IV. Results

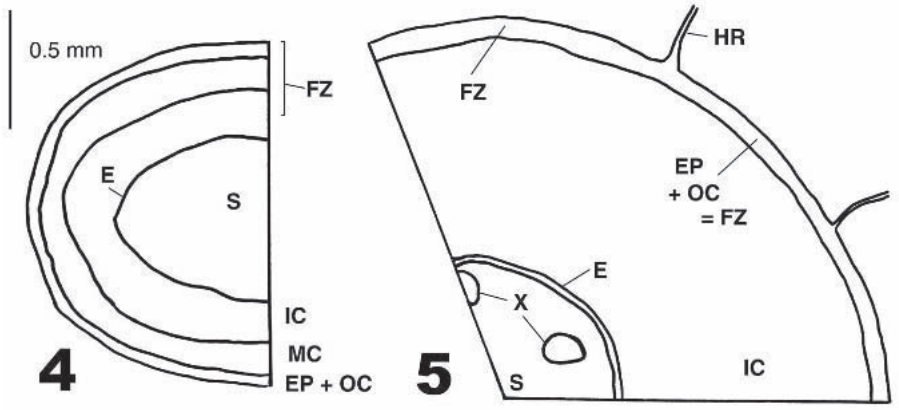
A. ROOT ANATOMY

In *Zamia* the ultimate fine roots, presumably the feeder roots, of the young plants used are third- or fourth-order roots. The first-order root is the main taproot of the seedling. Some apogeotropic fine second-order roots arise on the surface of the swollen first-order root and grow upward to produce coralloid root masses at the soil surface. Ultimate fine roots are brittle and easily break off when the soil is disturbed. Most were collected after being detached during the unpotting of the plants. All roots usually lack root hairs; only occasional irregular patches having root hairs. Growing roots have a defined root cap, and older roots have a periderm.

The ultimate roots have typical root structure. Most have two protoxylem points (diarch) and two groups of tracheary elements separated by a parenchymatous center (Fig. 1). The two alternating phloem poles have gelatinous fibers. The pericycle is two (rarely one) celled, and the endodermis has suberized radial walls that do not become thickened. There are no transfer cells when the endodermis is mature. The cortex is 9–13 cells thick in *Zamia,* with the outer two or three cells having lignified and suberized walls. Large druse crystals and fibers are scattered in the cortex of *Zamia*. The single epidermis becomes lignified. Root hairs may form along short longitudinal regions of the surface, but most roots lack root hairs (Fig. 2).

In older, thickened ultimate and next-lower-order roots, secondary growth begins in the pericycle. Xylem elements and phloem fibers are scattered in a mass of unlignified parenchyma. Secondary growth disrupts the endodermis, the inner two-thirds of the cortex is crushed, and the outer third becomes lignified (or suberized) and corklike (Figs. 2, 3).

In *Dioon,* typical root structure is found in young thick roots (first order) as well as older brittle second- and third-order roots. Only few scattered root hairs are present. In younger roots (Fig. 4) the epidermis and outer cortex is 3–4 cells thick, is slightly lignified, and stains darkly by trypan blue. The inner cortex is uniform and 14–21 cells thick. Mucilage cells or ducts have



Figs. 4, 5. *Dioon edule,* transverse sections of different ultimate roots. **4.** Young ultimate second-order root, no secondary growth, fungal zone in scattered cells from inner cortex to epidermis. **5.** Young thick first-order root, no secondary growth, fungal zone in scattered cells of outer cortex and epidermis. *Key:* E = endodermis; EP = epidermis; FZ = fungal zone; HR = root hair; IC = inner cortex; MC = middle cortex; OC = outer cortex; S = stele; X = xylem. Both sections at the same magnification.

thickened, lignified walls. There are usually three (rarely four) protoxylem points with central parenchyma. The pericycle is 2–4 cells thick. The endodermis has unthickened radial walls.

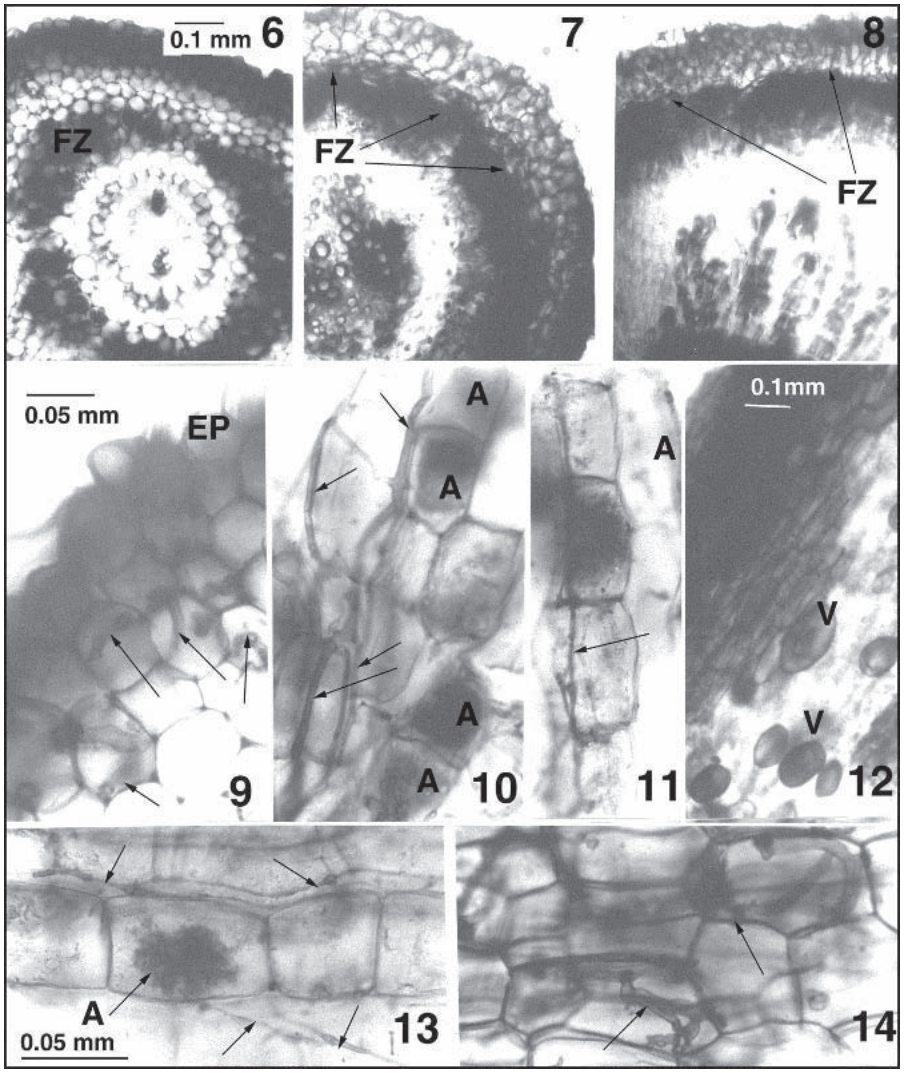
In older roots (Fig. 5), the cortex is clearly defined into outer, noncrushed, and inner, crushed, parenchyma. The epidermis and outer cortex is 8–10 cells thick, and the inner cortex consists of 8–10 thick-walled, crushed cells. The entire cortex is lignified. The endodermis is disrupted by secondary growth. Xylem elements and thick-walled phloem fibers are scattered in a mass of unlignified parenchyma. No druses were observed.

B. ARBUSCULAR MYCORRHIZAL FUNGI

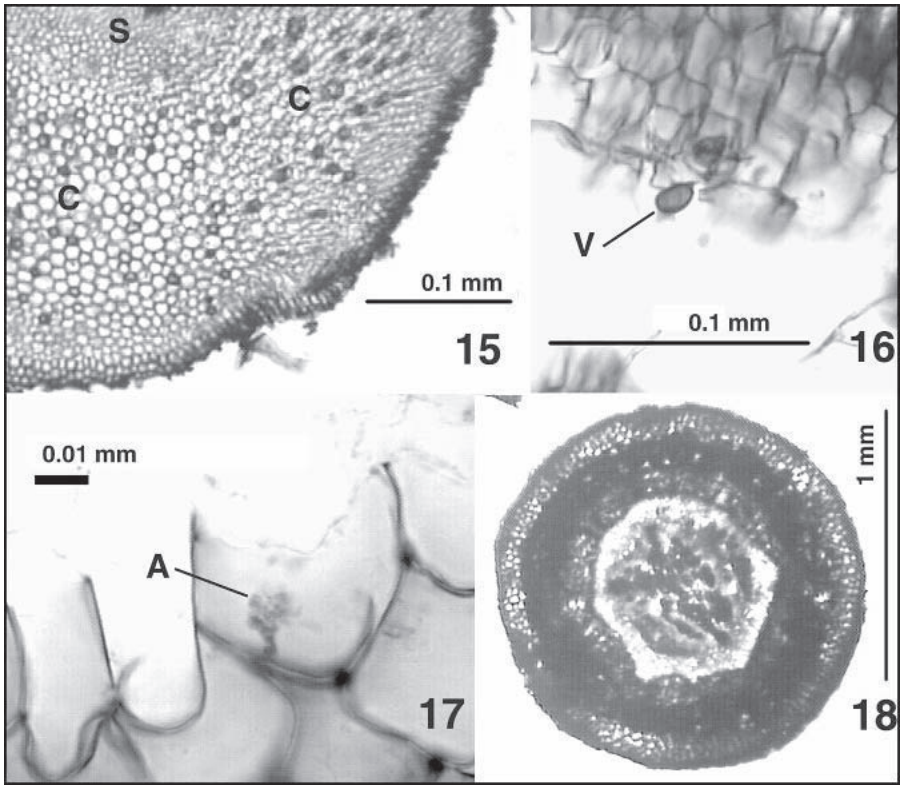
In *Zamia,* fungal hyphae (both septate and nonseptate) are on the surface of the root cap and on the epidermis and root-cap fragments farther behind the apex. At 5 mm behind the apex, nonseptate fungal hyphae occur within and between cells of the cortex (Figs. 1, 6). Hyphae occur similarly in regions with or without root hairs (Figs. 2, 7). Intercellular hyphae and arbuscules are abundant in the middle cortex (Figs. 10, 11, 13). Arbuscules arise from one or more branches of an external (intercellular) hypha (Fig. 13). The two (occasionally one) parenchyma cells next to the endodermis lack fungi and are followed by a 3–4-cell layer with fungi. Outside the fungal zone (Fig. 1) there are 2–3-cell layers of unlignified cortex, followed by 2–3-cell layers of lignified cortex and the epidermis.

Fungi penetrate the epidermal cells either at the center of the tangential wall or near a periclinal wall. Hyphae usually penetrate without a modified swelling or appressorium. The hypha forms one or more coils in the epidermal cell and the few radially adjacent cortical cells (Fig. 9) before spreading longitudinally in the root via intercellular spaces at the corners of cortical cells (Figs. 10, 11). Old roots contain cortical cells with internal, stubby-branched hyphae that appear to be remnants of former arbuscules (Fig. 14).

In older, thickened roots the cortex becomes crushed in the midregion from expansion of internal secondary tissues (Figs. 2, 3). Thus cell walls, hyphae, old arbuscules, and vesicles are



Figs. 6–14. *Zamia pumila,* sections of ultimate roots cleared and then stained with trypan blue. **6.** Transverse section of young root without secondary growth, fungal zone cells filled with arbuscules. **7.** Transverse section of older root with some secondary growth, fungal zone becoming crushed. **8.** Transverse section of older root with more secondary growth, fungal zone crushed. **9.** Transverse section of young root (as in Fig. 6), showing hyphal coils within cells of the epidermis and adjacent outer cortex. **10, 11.** Longitudinal sections of cells in the fungal zone (as in Fig. 6), with intercellular hyphae and arbuscules. **12.** Transverse section (as in Fig. 8), with vesicles in the crushed fungal zone. **13.** Longitudinal section (as in Fig. 6), with intercellular hyphae and arbuscules. **14.** Longitudinal sections (as in Fig. 7), with irregular intracellular hyphae that are interpreted as the remains of old arbuscules. *Key:* Arrows = hyphae; A = arbuscule; EP = epidermis; FZ = fungal zone; V = vesicle. Scale bars indicate magnification; Figs. 6–8 at the same magnification; Figs. 10, 11, 13, and 14 at the same magnification.



Figs. 15–18. *Dioon edule,* transverse sections of ultimate roots cleared and stained with trypan blue. **15.** Young root without secondary growth. **16.** Outer cortex of old root with vesicle near peripheral lignified parenchyma. **17.** Cortical parenchyma cell with arbuscule. **18.** Older root but with some secondary growth, the middle cortex crushed and darkly stained. *Key:* A = arbuscule; C = cortex; S = stele; V = vescicle. Scale bars indicate magnification.

compressed in a dark layer that easily detaches from the inner tissues during sectioning and handling. Vesicles and possibly spores are found in this region (Fig. 12). Fungi never occur within the endodermis or phellem outside the vascular cambium, or in the secondary vascular tissues, which consist mainly of parenchyma.

Young and old coralloid roots were sectioned at their unswollen bases and in their swollen regions containing *Nostoc*. Hyphae and other fungal structures were never seen in the coralloid roots, which were for the most part on or slightly above the soil surface.

In *Dioon,* very few arbuscular mycorrhizal fungi (AMF) were observed in roots. There was no clear fungal zone (Fig. 15) as in *Zamia,* perhaps as a consequence of the low colonization. Nonseptate hyphae and arbuscules occurred in cells of the outer, uncrushed cortex (Fig. 17) and epidermis. Although hyphae were found both between and within cells, the general pattern was arbuscules developing from intercellular hyphae.

Hyphae occur about 3 mm from the root tip. Vesicles and arbuscules occur in the matured regions (Fig. 16), about 12–15 mm from the tip. At 22 mm from the tip, hyphal clusters occur

within cortical cells and are interpreted as old arbuscules. Colonization by AMF is confined to the outer cortical cells (Figs. 4, 5). During secondary growth the middle cortex becomes crushed (Fig. 18), and cortex and AMF are lost.

In *Ceratozamia hildae,* roots taken from one individual had no evidence of AMF.

V. Discussion

A. ROOT ANATOMY AND AMF COLONIZATION

The thinnest feeder roots only irregularly form root hairs. The epidermis and outer cortex become suberized and lignified. Colonization by AMF proceeds from epidermal penetration, intracellular penetration in the outer cortical cells, and then longitudinal spread in the intercellular spaces of the cortex (middle in *Zamia;* outer in *Dioon*). AMF conform to the *Arum* type (Smith & Read, 1997; Smith & Smith, 1997), not the *Paris* type reported for many gymnosperms (Smith & Smith, 1997; McGee et al., 1999). As secondary growth develops, the fungal region of the cortex becomes progressively crushed, and vesicles and spore are present. Eventually the outer primary epidermis and cortex, including the fungal zone, are lost. Hyphae of AMF never penetrate the endodermis or stele. Fungal hyphae did not occur in coralloid roots in those cortical regions that lacked *Nostoc,* thus supporting similar observations of Joubert et al. (1989) in *Encephalartos*.

B. POSSIBLE SIGNIFICANCE OF MYCORRHIZAE

AMF enhance phosphorus uptake in low phosphorus soils, and this is especially important in legumes with nitrogen-fixing nodules (Smith & Read, 1997). In cycads, *Nostoc* fixes nitrogen when this cyanobacterium is present in specialized coralloid roots (Lindblad & Bergman, 1989; Lindblad et al., 1991). Thus AMF may well improve phosphorus uptake in cycads and promote the fixing of nitrogen by *Nostoc,* similar to the AMF effect on *Rhizobium* nodules in legumes. For example, *Macrozamia riedlei* has *Nostoc* and grows on infertile soils with very low levels of available phosphorus (1 ppm) in Australia. Grove et al. (1980) reported that *M. riedlei* in a jarrah forest ecosystem in southwestern Australia fixed ca. 35 kg of nitrogen per hectare in periods between successive burning of forests (5–7 years). Native habitats of *Zamia pumila* are also fire-maintained communities, in which available phosphorus is 6–9 ppm (Fisher & Jayachandran, in press). In the native habitat of *Dioon edule,* available phosphorus is 4.4 ppm (Vovides, 1999). Thus, future research should examine the interaction between AMF and *Nostoc* for nitrogen fixation in coralloid roots. Additionally, AMF may enhance water availability in both of these seasonally dry habitats, a benefit that has been reported in plants with poorly developed root hairs (Clarkson, 1974; Gerdemann, 1975; Smith & Read, 1997).

VI. Acknowledgments

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VII. Literature Cited

Brockhoff, J. O. & W. G. Allaway. 1989. Vesicular-arbuscular mycorrhizal fungi in natural vegetation and sand-mined dunes at Bridge Hill, New South Wales. Wetlands 8: 47–54.

Brundrett, M. C. & L. K. Abbott. 1991. Roots of jarrah forest plants, I. Mycorrhizal associations of shrubs and herbaceous plants. Aust. J. Bot. 39: 445–457.

———, **N. Bougher, B. Dell, T. Grove & N. Malajczuk.** 1996. Working with mycorrhizas in forestry and agriculture. Aust. Cent. Int. Agr. Res. Monograph 32: 1–374.

Clarkson, D. T. 1974. Ion transport and cell structure in plants. McGraw-Hill, London.

Fisher, J. B. & K. Jayachandran. 1999. Root structure and arbuscular mycorrhizal colonization of the palm *Serenoa repens* under field conditions. Plant Soil 217: 229–241.

Gerdemann, J. W. 1975. Vesicular-arbuscular mycorrhizae. Pp. 575–591 *in* J. G. Torry & D. T. Clarkson (eds.), The development and function of roots. Academic Press, London.

Grove, T. S., A. M. O'Connell & N. Malajczuk. 1980. Effects of fire on the growth, nutrient content and rate of nitrogen fixation of the cycad *Macrozamia riedlei*. Aust. J. Bot. 28: 271–281.

Joubert, L., N. Grobbelaar & J. Coetzee. 1989. *In situ* studies of the ultrastructure of the cyanobacteria in the coralloid roots of *Encephalartos arenarius, E. transvenosus* and *E. woodii* (Cycadales). Phycologia 28: 197–205.

Lindblad, P. & B. Bergman. 1989. Occurrence and localization of phycoerythrin in symbiotic *Nostoc* of *Cycas revoluta* and in the free-living isolated *Nostoc* 7422. Plant Physiol. 89: 783–785.

———, **C. A. Atkins & J. S. Pate.** 1991. N_2-fixation by freshly isolated *Nostoc* from coralloid roots of the cycad *Macrozamia riedlei* (Fisch. ex Gaud.) Gardn. Plant Physiol. 96: 753–759.

McGee, P. A., S. Bullock & B. A. Summerell. 1999. Structure of mycorrhizae of the Wollemi pine (*Wollemia nobilis*) and related Araucariaceae. Aust. J. Bot. 47: 85–95.

Norstog, K. J. & T. J. Nicholls. 1997. The biology of the cycads. Cornell Univ. Press, Ithaca, NY.

Reddell, P., M. S. Hopkins & A. W. Graham. 1996. Functional association between apogeotropic aerial roots, mycorrhizas and paper-barked stems in a lowland tropical rainforest in North Queensland. J. Trop. Ecol. 12: 763–777.

Smith, F. A. & S. E. Smith. 1997. Tansley Review No. 96. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. New Phytol. 137: 373–388.

Smith, S. E. & D. J. Read. 1997. Mycorrhizal symbiosis. Ed. 2. Academic Press, San Diego, CA.

Vovides, A. P. 1991. Vesicular-arbuscular mycorrhiza in *Dioon edule* Lindl. (Zamiaceae, Cycadales) in its natural habit in central Veracruz, Mexico. Brenesia 35: 97–103.

Note added in proof: *Arum*-type arbuscular mycorrhizae were reported in two species of *Cycas* and one unidentified species of *Zamia* (Muthukumar, T. & K. Udaiyan. 2002. Arbuscular mycorrhizas in cycads of southern India. Mycorrhiza 12: 213–217.) after our article was in press.