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Presence of arbuscular mycorrhizal fungi in South Florida native plants

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Abstract The roots of 27 species of South Florida plants in 15 families (including one cycad, six palms, one *Smilax*, and 19 dicotyledons) native to pine rockland and tropical hardwood hammock communities were examined for arbuscular mycorrhizal fungi (AMF). These plants grow in the biologically diverse but endangered Greater Everglades habitat. Roots from field-grown and potted plants were cleared and stained. All 27 species had AMF and include 14 species having an endangered or threatened status. The *Paris*-type colonization occurred in two species in the families Annonaceae and Smilacaceae. The *Arum*-type occurred in 22 species in the families Anacardiaceae, Arecaceae (Palmae), Boraginaceae, Cactaceae (questionable), Euphorbiaceae, Fabaceae, Lauraceae, Melastomataceae, Polygalaceae, Rubiaceae, Simaroubaceae, Ulmaceae, and Zamiaceae. Three species in the families Fabaceae, Lauraceae, and Simaroubaceae had a mix of *Paris*- and *Arum*-types. The results have implications for the restoration of these endangered plant communities in the Everglades.

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Introduction

The restoration of threatened ecosystems and the reestablishment of endangered plants should be based on a sound biological understanding of the plants. A great effort is now under way to save and restore the Everglades ecosystem of South Florida, USA. Future restoration of keystone species and introduction of rare species on degraded sites will require detailed biological information on the separate component species. In southeastern Florida, a significant part of the natural vegetation grows on upland sites that are raised only a meter or two above the more widely known wetlands of the Everglades. These upland plant communities contain many endangered plants (seven federally listed and numerous state listed), which grow on shallow sandy soils on top of a limestone base (U.S. Fish and Wildlife Service 1999; Coile and Garland 2003). The flora is a fascinating mix of northern temperate elements (*Pinus*, *Quercus*, and *Rhus*) and tropical elements (palms, tropical dicots, and a cycad).

Originally, there were two subtropical forest types: pine rockland and hardwood hammock (Wunderlin and Hansen 2000) in the nonflooded sites. Because of urbanization (metropolitan Miami) and agricultural expansion, these two subtropical forest types are now highly threatened by habitat loss, with less than 5% remaining outside Everglades National Park. Federal, state, and local land managers are working to protect the few remaining fragments of these habitats. They seek to restore numerous endemic plants as part of a multispecies recovery plan for the Greater Everglades region (U.S. Fish and Wildlife Service 1999).

An important aspect of seedling establishment and survival on shallow infertile soils, as occur in these habitats, is the relationship between their roots and soil microorganisms and, in particular, mycorrhizal fungi, which are ubiq-

uitous and have been shown to be significant biotic variables in many habitat restorations elsewhere (Pattinson et al. 2004; Sylvia et al. 1993; Smith and Read 1997). However, surprisingly little information on mycorrhizal associations in South Florida vegetation types has been published, considering the international recognition of the Everglades Biosphere as a World Heritage Site (http://www.unesco.org/mab) and the national commitment of money and effort in its conservation and restoration. Arbuscular mycorrhizal fungi (AMF) were reported in some Everglades wetland species by Aziz et al. (1995) and Jayachandran and Shetty (2003), and we have reported on AMF in three pine rockland species (Fisher and Jayachandran 1999, 2002; Fisher and Vovides 2004). However, the significance of AMF for such a diverse and endemic flora cannot be determined from such a small sampling. The fungal morphology was described in only three of these plant species from the Everglades. More upland plants need examination for AMF, and this information will serve as a foundation for future experimental research on the effectiveness of AMF on seedling growth of these plants.

The diversity of this subtropical flora also offers an opportunity to expand our knowledge of the morphology of AMF in roots of 27 plant genera in 15 families. Basic information on the taxonomic distribution of *Arum*- and *Paris*-types in plants (Smith and Smith 1997) may help clarify the continuing debate about the influence of the plant, the fungus, or both on the morphology of the association, as recently discussed in several papers (Ahulu et al. 2005; Cavagnaro et al. 2001; Dickson 2004; Kubota et al. 2005; Yamato 2004).

Materials and methods

Soil

Native soil was collected from a remnant of natural pine rockland vegetation in Miami-Dade County, FL. The following plants grow within 10 m of the collection site: *Bursera simaruba* (L.) Sarg., *Myrica cerifera* L., *Pithecellobium keyense* Britton ex Britton & Rose, *Quercus* spp., *Rhus copallinum* L., and *Serenoa repens* (W. Bartram) Small. The site has shallow sandy soils over a bed of oolitic limestone. The surface 10 cm is a white sand (pH=6.2–6.8; available P=5.9–8.8 mg kg^{-1}) (bicarbonate extraction; Bray and Kurtz 1949). The soil at 20–50 cm depth in deep pockets is a yellow-orange sand (pH=6.3–8.2; available P=11.5–17.3 mg kg^{-1}). Soil from surface and deeper soil profiles of this one natural pine rockland area was mixed together to give a final available P=ca. 10.0 mg kg^{-1} soil. We also determined the level of soil-solution P in water extraction through the methods of Olsen and Summers (1982). Mixed fresh native soil samples from 0- to 20-cm depths had an average soil-solution P=0.0021 mg l^{-1} (SE 0.0011, n=4). Soil was sieved through 6-mm mesh to remove stones and large root fragments, stored in plastic tubs at 20–22°C, and used within 1 week.

Plant materials

Since the ultimate feeder roots of wild plants were difficult to extract from the limestone rock, we planted sterile seedlings in nurse culture pots, similar to those used for AMF inoculum, to examine the inoculum potential and morphological types formed. If possible, field-collected roots were also examined. This use of seedlings was similar to the method of Brundrett and Abbott (1991). Seeds were collected from cultivated plants in the ex situ conservation collection at Fairchild Tropical Botanic Garden (Coral Gables, FL) or from wild native plants at this site and nearby Montgomery Botanical Center and USDA/ARS Station (see Table 1 for full nomenclature of species). Because *Consolea corallicola* does not set seed, small pads were collected from mature plants and rooted in AMF-free medium of pure Perlite.

AMF inoculum

Soil (5–15 cm depth) and root fragments of palms and dicots were collected from a site of natural pine rockland vegetation and placed in 2.5-l pots. Nurse cultures of native AMF were maintained in a greenhouse and renewed with fresh soil. Host plants were pigeon pea [*Cajanus cajan* (L.) Millsp.] and Sudan grass [*Sorghum arundinaceum* var. *arundinaceum* (Desv.) Stapf], grown together in the same pot. Nurse cultures were at least 12 weeks old before use. The inoculum samples showed heavily colonized root fragments and numerous AMF spores. Soil and root fragments were mixed well and used as mixed AMF inoculum. Spores of many morpho-species of *Glomus*, *Gigaspora*, *Scutellospora*, and other Glomalean-like fungi were recovered from sieved inoculum samples. Although we assume that inoculum from nurse cultures represents the range of AMF propagules found in the original habitat (Klironomos and Hart 2002), it is probable that not all the same AMF colonize the 27 host species that we examined, as was shown by variation in AMF trapped by different host plants (Liu and Wang 2003). Similarly, the two host plants species may not be colonized by all AMF present in the original soil samples.

AMF colonization

Each root sample was cleared in KOH, bleached with ammoniated H_2O_2, and stained with trypan blue or chlorazol black E in acidic glycerol (Brundrett et al. 1996) to reveal the presence of AMF. The basic morphology of the AMF colonization was classified as *Arum*- or *Paris*-type, based on whether fungal hyphae were present mainly between cells as hyphae running through intercellular spaces or within the cells as coils, respectively, following the descriptions of Smith and Smith (1997) and expanded upon by Dickson (2004). Since we examined whole and squashed roots, we could not reliably distinguish among interme-

Table 1 List of taxa with their families, plant community type, and observations on arbuscular mycorrhizal fungi (AMF) colonization

Taxon	Status[a]	Family	Plant community	Source of roots[b]	AMF description					
					Cortical AMF hyphae	Coils	Vesicles	Arbuscules	Root hairs	AMF type[c]
Dicotyledons										
Annona glabra L.		Annonaceae	Coastal hammock (wet edge), wetlands	P	+	+	None	None	None	*Paris*
Bourreria cassinifolia (A. Rich.) Griseb.	E	Boraginaceae	Pine rockland	P	+	+Few	+	+?	+Short	*Arum*
Consolea corallicola Small (*Opuntia spinosissima* Mill.)	E	Cactaceae	Coastal hammock	P	+	None	+	None	+Long (0.5 mm)	*Arum*?
Erithalis fruticosa L.	T	Rubiaceae	Coastal hammock	P	+	+Rare	+	+	+	*Arum*
Gymnanthes lucida Sw.		Euphorbiaceae	Coastal hammock	P	+	None	None	+	+	*Arum*
Hamelia patens Jacq.		Rubiaceae	Pine rockland, coastal hammock	F, P	+	+Rare	+	+	+	*Arum*
Harrisia fragrans Small ex Britton & Rose	US	Cactaceae	Coastal hammock	P	+Rare	None	None	None	+Long (0.8–1.2 mm)	*Arum*?
Licaria triandra (Sw.) Kosterm.	E	Lauraceae	Coastal hammock	P	+	+Mixed	None	+	None	*Arum* +*Paris*
Ocotea (*Nectandra*) *coriacea* (Sw.) Griseb.		Lauraceae	Coastal hammock	F, P	+Rare	None	+	None	None	*Arum*
Opuntia tricanthos (Willd.) Sweet	E	Cactaceae	Coastal hammock	P	+?	None	None	None	?(+)	*Arum*?
Picramnia pentandra Sw.		Simaroubaceae	Coastal hammock	F, P	+Few inter-cellular	+	+Within cell	+	+	*Arum* +*Paris*
Polygala smallii R.R. Sm. & D.B. Ward	US	Polygalaceae	Pine rockland	F	+	None	+	+	None	*Arum*
Psychotria nervosa Sw.		Rubiaceae	Pine rockland, coastal hammock	F, P	+	+Rare	+	None	+Short, rare	*Arum*
Rhus copallinum L.		Anacardiaceae	Pine rockland	F, P	+	None	+	+	+	*Arum*
Simarouba glauca DC.		Simaroubaceae	Hammock	F, P	+	+	+	+	+Rare, short	*Arum*
Sophora tomentosa L. var. *truncata* Torr. & A. Gray		Fabaceae (Papilionoideae)	Pine rockland, hammock	P	+	+	+	+	+	*Arum* +*Paris*?
Tephrosia angustissima var. *corallicola* (Small) Isely	E	Fabaceae (Papilionoideae)	Pine rockland	F, P	+	None	+	+	+	*Arum*
Tetrazygia bicolor (Mill.) Cogn.	T	Melastomataceae	Pine rockland, hammock	P	+	+Rare	+	+	+Rare, long	*Arum*
Trema micranthum (L.) Blume		Ulmaceae	Pine rockland, hammock	P	+	None	+	+	+	*Arum*
Palms										
Acoelorrhaphe wrightii (Griseb. & H. Wendl.) H. Wendl. ex Becc.	T	Arecacae (Palmae)	Coastal hammock (wet edge), wetlands	F, P	+	None	+	+	None	*Arum*
Coccothrinax argentata (Jacq.) L.H. Bailey	T	Arecacae (Palmae)	Pine rockland	F, P	+	+(Near epidermis)	None	+	None	*Arum*

Table 1 (continued)

Taxon	Status[a]	Family	Plant community	Source of roots[b]	AMF description					AMF type[c]
					Cortical AMF hyphae	Coils	Vesicles	Arbuscules	Root hairs	
Pseudophoenix sargentii H. Wendl. ex Sarg.	E	Arecaceae (Palmae)	Coastal hammock	F, P	+Dense network	None	+	+	+Rare	*Arum*
Sabal palmetto (Walter) Lodd. ex Schult. & Schult.f.		Arecaceae (Palmae)	Pine rockland, coastal hammock	F, P	+	+Rare	+	+	None	*Arum*
Serenoa repens (W. Bartram) Small		Arecaceae (Palmae)	Pine rockland	F, P	+	+Near epidermis	+	+	None	*Arum*
Smilax havanensis Jacq.	T	Smilacaceae	Pine rockland	F, P	+	+Multiple	None	+	+Short	*Paris*
Thrinax morrisii H. Wendl.	E	Arecaceae (Palmae)	Pine rockland, coastal hammock	F, P	+	None	+	+	None	*Arum*
Cycad										
Zamia pumila L.		Zamiaceae	Pine rockland	F, P	+	None	+	+	+	*Arum*

[a]Endangered status: *E* State of Florida endangered, *T* State of Florida threatened (Coile and Garland 2003), *US* federally endangered (U.S. Fish and Wildlife Service 1999)
[b]Observed roots from: *F* field grown plants, *P* potted nurse cultures
[c]? Uncertainty about AMF presence in root

diate morphologies as described and classified by Dickson (2004).

Results

AMF colonization

Some roots, especially those of palms, were difficult to clear and stain because of thick, lignified walls of epidermis and hypodermis. For these plant species, AMF could only be observed reliably in thick transverse or oblique longitudinal sections that were processed like whole-root fragments or after longitudinal splitting and dissection of the root cortex. Ultimate short fine roots of palms, *Zamia pumila*, and several dicots were brittle and easily detached during digging and removal of soil. This was particularly apparent when roots of these plants were initially excavated in the field. The fine roots of palms and some dicots had distinctive surface structure and color, and were observed detached in loose soil during field excavation in the field. Few fine roots possessing AMF were collected directly from naturally shallow, rocky soils. Occasionally, we found a proliferation of fine roots in a small pocket of humus or in deep crevices in the limestone substrate. In these sites, AMF were abundant but absent in roots of the same plant extracted elsewhere. For these reasons, we relied on pot (also called trap) cultures to assess mycorrhizal status and morphologies, although roots derived from both field and pots were examined, as noted in Table 1. Most seedlings growing in pots with AMF inoculum showed AMF colonization after 8 weeks or longer.

Dicotyledons

The presence of root hairs was variable (Table 1). In most cases, features that are typical of *Arum*-type colonization (Smith and Smith 1997) were found. Arbuscules were mainly found in younger regions of roots, typically one per cortical cell (Fig. 1a). Nonseptate hyphae were mostly found in the longitudinal intercellular spaces of the root cortex (Fig. 1b,c). Intercellular hyphae proliferated in deeper layers of cortex and not in the epidermis or peripheral layers that were adjacent to the region of hyphal penetration (Fig. 1a, c). Vesicles were found in older regions of roots (Fig. 1d).

Four dicot species had fungal morphology that was typical of the *Paris*-type (Smith and Smith 1997) in which neighboring cortical cells contained hyphal coils without hyphae in the intercellular spaces. Three of these dicots had a mix of AMF morphology. *Picramnia pentandra* had intracellular hyphal coils and a single arbuscule per adjacent cortical cell (Fig. 2a, b), but typical *Arum*-type in other roots of the same plant. One root of *Licaria triandra* was observed with *Paris*-like, highly coiled hyphae (several coils per cell) in the periphery of the cortex in one region, yet another region of the same root formed a longitudinal intercellular network of hyphae connecting cells with one

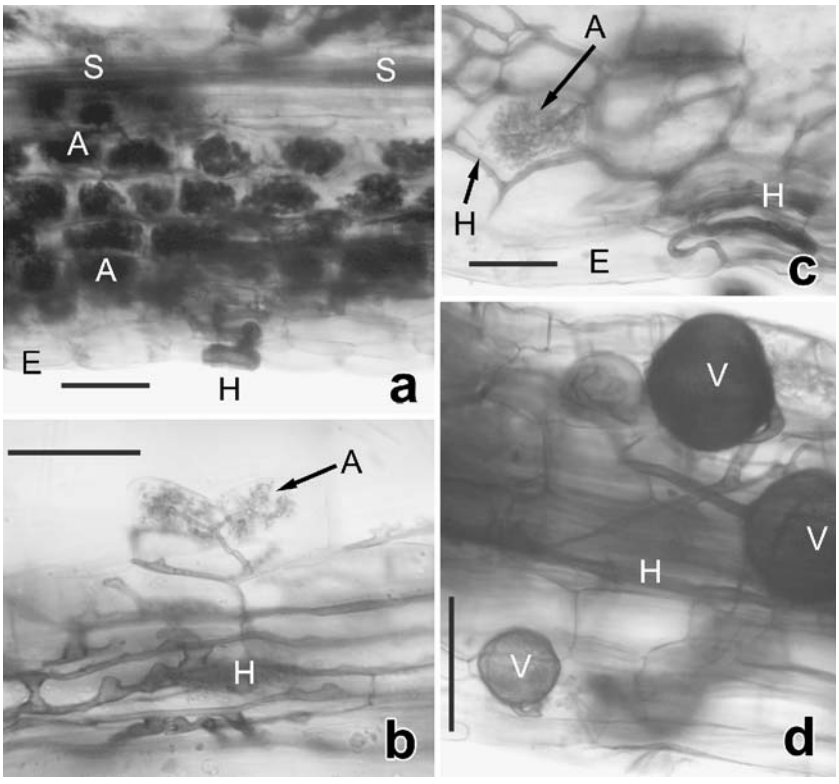

Fig. 1 Arbuscular mycorrhizal fungi in cleared roots stained with trypan blue. **a** *Tetrazygia bicolor*, arbuscules fill the cortex parenchyma cells and penetration hypha is in the epidermis and hypodermis. **b**, **c** *Simaruba glauca*, arbuscules in cortical parenchyma with longitudinal intercellular hyphae. Penetration hypha in epidermis of **c**. **d** *Hamelia patens*, vesicles and intercellular hyphae. *A* arbuscule, *E* epidermis, *H* hypha, *S* stele of root, *V* vesicle; all *bars* 50 μm

arbuscule per cell, typical of the *Arum*-type. *Sophora tomentosa* var. *truncata* had features of both *Arum*- and *Paris*-types in different roots of the same plant. Only *Annona glabra* had consistently cortical parenchyma cells with multiple hyphal coils within each cell and no intercellular hyphae.

Hyphal coils were rarely observed in the outer regions of the cortex of *Ocotea coriaceae*, *Psychotria nervosa*, *Simarouba glauca*, and *Tetrazygia bicolor*. Because the coils were not sufficiently common and most hyphae were intercellular, we classify these plants as *Arum*-type (Table 1).

All three species of cactus (*C. corallicola*, *Harrisia fragrans*, and *Opuntia tricanthos*) had noticeably long root hairs (0.5–1.2 mm long) that clung to sand particles and made a sand sheath around the root. Although root surfaces had both septate and nonseptate hyphae, only rarely were hyphae found in longitudinal intercellular spaces and appeared to be nonseptate. Neither arbuscules nor vesicles were observed. We were unsure if these plant species had AMF when grown in pots. Therefore, the AMF type is noted with a question mark in Table 1. Roots of *Consolea* and *Harrisia* formed root knots caused by nematodes, which were observed in the roots. We were unable to collect and observe roots from wild plants.

Monocotyledons

All six species of palm had lignified, rough (papilliform), thick-walled epidermal cells that made staining and clearing difficult. They were best observed in thick transverse or longitudinal sections of ultimate roots. Root hairs were not found. All ultimate fine roots were brittle and easily detached during excavation and cleaning.

Coccothrinax argentata had a dense network of intercellular hyphae in the cortex (Fig. 2d). Both intercellular and intracellular coils formed in the many-layered hypodermis (Fig. 2e), and a single arbuscule per cell (Fig. 2f) and vesicles were found. *Sabal palmetto* and *S. repens* had dense intercellular hyphae in the cortex periphery with arbuscules and vesicles, but they also had coils in the epidermis and hypodermal cells. Coils were not observed in *Acoelorrhaphe wrightii*, *Pseudophoenix sargentii*, and *Thrinax morrisii*.

Smilax havanensis had cortical cells filled with multiple hyphal coils without obvious intercellular hyphae (Fig. 2c). The hyphal coils in adjacent cells were connected by a single hypha that passed through the common cell wall. Occasionally, arbuscules were found in one or two cells distant from coils, rarely adjacent to coils. These arbuscules were simple, with a single-trunk hypha and were not definitely associated with the coils within the same cell. *Smilax* displayed the classic *Paris*-type.

Cycad

Z. pumila had typical *Arum*-type AMF with longitudinal intercellular hyphae and arbuscules concentrated in the outer cortex. Vesicles formed in older roots, especially after secondary growth was present in the root.

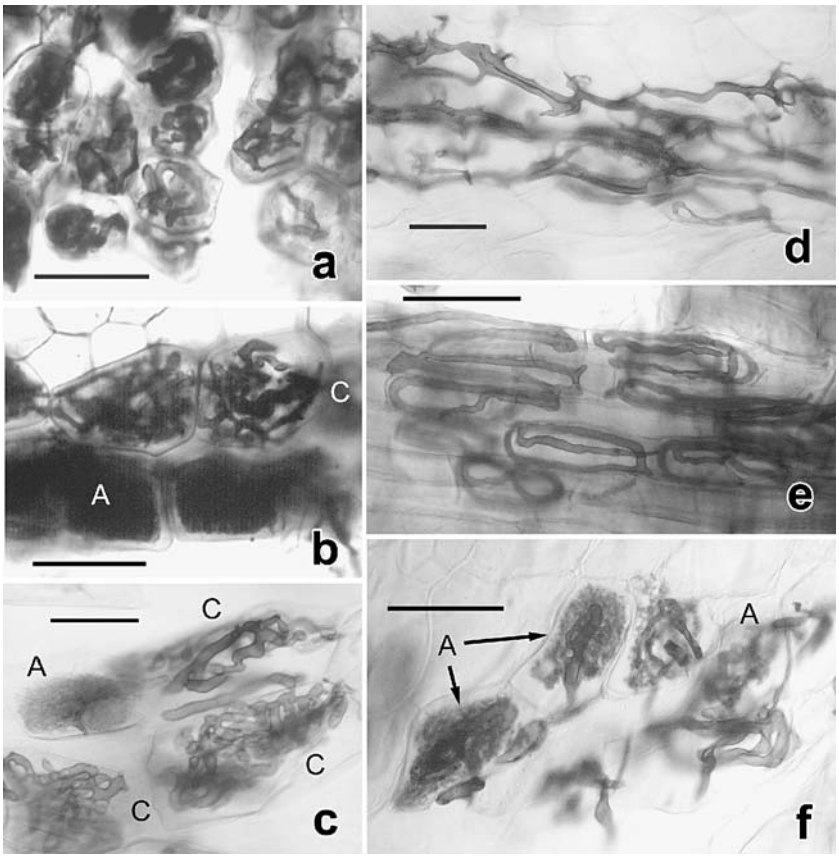

Fig. 2 Arbuscular mycorrhizal fungi in cleared roots stained with trypan blue. *Picramnia pentandra*, hyphal coils fill cortical parenchyma cells in **a**, coils in parenchyma cells and single arbuscules in adjacent cells in **b**. **c** *Smilax havanensis*, coils in cortical parenchyma with single arbuscule in adjacent cell. *Coccothrinax argentata*, intercellular hyphae in **d**, intracellular coils in hypodermal and outer cortical cells in **e**, arbuscules in cortical parenchyma cells in **f**. *A* arbuscule, *C* hyphal coil; all *bars* 50 μm

Discussion

AMF colonization

Of the 27 species growing in the wild or exposed to AMF in pot culture, 24 formed clearly defined arbuscular mycorrhizae (AM) and three cacti (*Consolea*, *Harrisia*, and *Optuntia*) had poorly developed or uncertain AM. The lack of clear AMF structures in these cacti was unexpected because AMF colonization was reported in other cacti (Allen et al. 1998; Barredo-Pool et al. 1998; Carrillo-Garcia et al. 1999) and also increased growth and P uptake (Pimienta-Barrios et al. 2002; Rincón et al. 1993). However, Pimienta-Barrios et al. (2003) found that when a fungicide was applied to eliminate mycorrhizal colonization in natural plants of *Opuntia robusta*, physiological processes were unaffected. The inhibition of AMF colonization by benomyl did not affect photosynthesis, water uptake, or P uptake under prolonged drought. Interestingly, the three cacti we examined had noticeably long root hairs that formed sand sheaths, which required extra effort in freeing roots from soil particles. The other plant species had few or no root hairs (Table 1). This observation supports the loose relationship between AM and relatively thick feeder roots with short or no hairs, so-called magnolioid roots (Smith and Read 1997; Fitter 2004). However, the lack of AMF in the three cacti, which have long root hairs, could have been caused by the inhibition of AMF colonization due to pathogens or parasites (nematodes) in the mixed inoculum, since we observed root knot nematodes in some cactus roots.

Many of the 24 noncactus species were found with AMF in nature. We presume that those roots that did not have clear AMF in nature or had very low rates of colonization may have been artifacts of the difficulty in extracting fine feeder roots from the rocky substrate. Mycorrhizal status of plant species, growing in this substrate in which roots tend to proliferate in rock crevices and at great distances from the shoot, is best determined with trap cultures. We found that feeder roots containing AMF may possibly be lost during extraction or missed when roots proliferate at localized nutrient-rich or moist sites, a characteristic of feeder roots noted by Hodge (2004). Olsson et al. (2002) showed that humus-rich soil or organic matter promote or enhance mycorrhizal proliferation. This is the likely reason that the first survey of mycorrhizae in South Florida did not find AMF in roots collected in nature for many of these same species (Meador 1977). Later investigations did report AMF in wetland plants of the Everglades (Aziz et al. 1995; Jayachandran and Shetty 2003), plants of the pine rocklands (Fisher and Jayachandran 2002; Fisher and Vovides 2004), and plants of coastal dunes (Fisher and Jayachandran 2002; Sylvia et al. 1993).

The two main structural types of AM in host roots were reviewed by Smith and Smith (1997): the *Arum*-type with intercellular hyphae in the root cortex; and the *Paris*-type with intracellular hyphal coils and no intercellular hyphae. Our survey found that 21 species formed the *Arum*-type

and expands the categorization of AM according to colonization type as reviewed in Smith and Smith (1997). Previously, we reported that *Amorpha crenulata* (Fabaceae, Papilionoideae) form *Arum*-type morphology (Fisher and Jayachandran 2002). Details of root anatomy and *Arum*-type AMF were described in *Zamia* (Fisher and Vovides 2004) and *Serenoa* (Fisher and Jayachandran 1999). In all the above plants, we observed that many of the species had some intracellular hyphal coils in the epidermis and hypodermis, often near the point of hyphal penetration of the root. However, most hyphae occur in intercellular spaces deeper in the cortex. This variation has been reported widely in the literature and was classified as the *Arum*-type by Smith and Smith (1997), but may be the cause of some apparent conflicting reports of *Arum*- versus *Paris*-types depending upon interpretation of the intracellular hyphal coils that occur in the outer cortex. A distinct hypodermis is often absent in feeder roots of some species, as in the fifth-order roots of the palm *Serenoa* (Fisher and Jayachandran 1999). Within the inner cortex of the same roots, extensive networks of intercellular hyphae formed arbuscules and vesicles. Since Smith and Smith's review, Wubet et al. (2003) found *Arum*-type in all 11 indigenous trees in Ethiopia, although they report rare hyphal coils near the points of new infection. More typical *Arum*-type hyphae in the intercellular spaces of inner cortex may be poorly developed or are not yet present in a particular root being observed; i.e., the problem of small sample size in any study. Among plants in a mangrove community, Sengupta and Chaudhuri (2002) reported 12 *Arum*-types, 27 *Paris*-types, and 13 with both types. Yamato (2004) found that 20 species of plants (out of 26) growing in an old field had *Arum*-type AM with intermediate types in Poaceae. However, Dickson (2004) found that *Paris*- and *Arum*-types represent the extremes of a range of morphologies and emphasized the difficulty in using a simple classification scheme (as noted below). Thus, the classification of *Arum*- versus *Paris*-type may vary depending upon the interpretation of fungal structures by the investigator.

Two species in our study (*Annona* and *Smilax*) formed only intracellular coils typical of the *Paris*-type colonization. We confirm the description of AMF in *Smilax* by Maremmani et al. (2003) and Bedini et al. (2000), where a single arbuscule forms in each cell adjacent to cells filled with hyphal coils (as seen in Fig. 2c). Bedini et al. (2000) found that two species of *Glomus*, which produce *Arum*-type AM in other plant species, formed *Paris*-type in *Smilax*. Growth was also increased by AMF colonization. Their finding supports the general assumption that the host root mainly determines the type of AM structure, not the fungus (Smith and Smith 1997).

Three species (*Licaria*, *Picramnia*, and *Sophora*) formed both *Arum*- and *Paris*-types of AM within roots of the same plant using the same mixed inoculum as with all the other species. However, we do not know how many different AMF are associated with the different types of colonization, nor if the same or different AMF species cause the mixed *Arum–Paris* types in the same root system. We cannot state that these three plant species are "near-*Paris*" or "intermediate types" (Smith and Smith 1997; Dickson 2004) because of the possibility that more than one AMF is involved in each symbiosis. It has been generally assumed that the AMF structure is in great part regulated by the plant; each plant species has a particular type of colonizing fungal morphology, as seen in the findings of Bedini et al. (2000) described above. Nevertheless, in tomato, the AMF-type varied depending upon the AMF species, thus indicating a combined fungal and plant control of morphology (Cavagnaro et al. 2001). This observation was extended by Kubota et al. (2005), who found that both types were induced in tomato and cucumber but only the *Paris*-type in *Clethra* by the same field-collected soil. Then, using fungal DNA from the roots, they found that AMF morphology was correlated with the fungal family present, as determined by specific DNA primers.

A recent detailed comparison of six AMF on 12 host plants has documented that fungal morphology is affected by both partners (Dickson 2004). Intermediates between *Arum*- and *Paris*-types were described in detail. We are presently establishing cultures of single native AMF species which will be used in the future to clarify this point in the plant species we surveyed (Table 1). If two or more AMF species are involved in our examples of mixed types, it will also be interesting to document whether the AMF complement one another in their benefit to the plant's nutrition as has been suggested (Sanders 2002) and recently documented using two *Glomus* species in the same host plant (Drew et al. 2003). The speed and amount of colonization of roots by AMF also varies with the fungal species and seems related to family classification of the fungus (Hart and Reader 2002). Future research must clarify the identity of the AMF species involved in South Florida soils.

Significance for conservation and ecology

All the species examined (except uncertainty for the three cacti) were colonized by AMF, which was expected in the two natural habitats with shallow, sandy, and nutrient poor soils. Of these 27 plants, 14 have endangered or threatened status (Table 1). Knowing that plants of conservation concern are mycorrhizal is important when developing the best methods for growing plants used in reintroductions, as emphasized by Gemma et al. (2002), Fisher and Jayachandran (2002), Fuchs and Haselwandter (2004), Koske and Gemma (1995), and Pattinson et al. (2004). The next phase of our research will entail experimental studies of these plant species to determine the effects of AMF inoculation on growth. We expect that some, if not all, species will require AMF for successful seedling establishment and reproduction in natural habitats.

Yamato (2004) has suggested a relationship between AMF morphology and the ecology of the plant. He found a predominance of the *Arum*-type in the weedy herbs and vines growing in an old abandoned field. He interpreted his and an earlier work (e.g., Brundrett and Kendrick 1990) as evidence that *Arum*-type may be more frequent in fast-

growing plants, and the *Paris*-type more common in understory, slow-growing herbs. Ahulu et al. (2005) also found weak correlations between AMF morphology and the growth habit and successional status of 35 plant species from the same community. We cannot interpret our findings in this respect because the herbaceous understory species were not well represented in our survey.

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