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DIVISÃO 2 - PROCESSOS E PROPRIEDADES DO SOLO

Comissão 2.1 - Biologia do solo

ARBUSCULAR MYCORRHIZAL INOCULATION INCREASES BIOMASS OF *EUTERPE EDULIS* AND *ARCHONTOPHOENIX ALEXANDRAE* AFTER TWO YEARS UNDER FIELD CONDITIONS[1]

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SUMMARY

Inoculation with arbuscular mycorrhizal fungi (AMF) of tree seedlings in the nursery is a biotechnological strategy to improve growth, survival after transplanting, biomass production and to reduce the use of fertilizers. *Archontophoenix alexandrae* and *Euterpe edulis* are palm species used in southern Brazil to produce the palm heart, the latter being included in the list of threatened species due to the overexploitation of its native population. The purpose of this paper was to evaluate the effect of mycorrhizal inoculation on growth and physiological parameters of *A. alexandrae* and *E. edulis*. After germination, the seedlings were inoculated (AMF) or not (CTL) with AMF in the treatments. Values of chlorophyll content, biomass and shoot phosphorus were not statistically different between the AMF and CTL treatments, after five months in the greenhouse. Inoculation with AMF significantly increased the levels of starch and soluble carbohydrates in shoots and roots of both species. Under field conditions, AMF had no effect on stem diameter and height after 12 and 24 months, but total plant biomass and leaf, stem and root biomass were greater in AMF than in CTL plants. The data indicated that AMF inoculation in the nursery

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has a strong effect on biomass accumulation after growing for 24 months under field conditions. Therefore, AMF inoculation should be considered an important strategy to increase growth and production of these economically important tropical palm species.

Index terms: palm species, soluble carbohydrates, plant carbon, field inoculation, allometric equations.

RESUMO: *INOCULAÇÃO MICORRÍZICA ARBUSCULAR AUMENTA BIOMASSA DE EUTERPE EDULIS E ARCHONTOPHOENIX ALEXANDRAE APÓS DOIS ANOS EM CONDIÇÕES DE CAMPO*

A inoculação de plântulas no viveiro com fungos micorrízicos arbusculares (FMAs) é uma estratégia biotecnológica para aumentar o crescimento, a sobrevivência após o transplantio, e a produção de biomassa e reduzir o uso de fertilizantes. Archontophoenix alexandrae *e* Euterpe edulis *são espécies de palmeiras usadas no sul do Brasil para produzir o palmito - a última espécie incluída na lista daquelas ameaçadas devido à superexploração das suas populações nativas. O objetivo deste artigo foi avaliar o efeito da inoculação micorrízica no crescimento e parâmetros fisiológicos de* A. alexandrae *e* E. edulis*. Após a germinação, as plântulas foram submetidas aos tratamentos de inoculação micorrízica (FMA) ou não inoculadas (CTL). Os conteúdos de clorofila, biomassa e fósforo da parte aérea não foram significativamente diferentes entre os tratamentos após cinco meses em casa de vegetação. A inoculação alterou significativamente os níveis de amido e carboidratos solúveis na parte aérea e nas raízes de ambas as espécies. Em campo, os FMAs não tiveram efeito no diâmetro do caule e na altura após 12 e 24 meses, porém a biomassa total e a biomassa de folhas, caule e raízes foram maiores em plantas inoculadas, comparadas com plantas controle. Os resultados indicam que a inoculação com fungos micorrízicos arbusculares no viveiro tem papel importante no acúmulo de biomassa após 24 meses de transplantio para o campo. Assim, a inoculação micorrízica deve ser considerada uma importante estratégia para aumentar o crescimento e a produção dessas espécies de palmeiras tropicais que possuem importância econômica e ambiental.*

Termos de indexação: palmeiras, carboidratos solúveis, carbono na planta, inoculação em campo, equações alométricas.

INTRODUCTION

Palm species (Family Arecaceae) are an important group of plants within the ecosystems they are distributed in, by providing shading to seedling populations sprouting on the forest floor and as an important food source in tropical forests, especially for bird species (Andreazzi et al., 2009). The economic value of palm species is high, as they are a source of fruits (*e.g.*, coconut), oils, fiber, and the palm heart (Joly, 2002).

Brazil accounts for 95 % of the world market of the palm heart. Of the exploited species, *Euterpe edulis* Mart. (Jussara palm) needs particular attention since its intensive exploitation has included it in the red list of species threatened by extinction. *E. edulis* is native to the tropical rainforest of the biome Atlantic Forest (Mata Atlântica) and considered a key species for sustainable forest management. This palm species has only one stem (height 10 - 20 m) and produces fruits for *ca.* 6 months per year; it is generally used as a food source of the wildlife, which in turn contributes to the population dynamics and gene flow in this species (Lorenzi et al., 2004; Martins-Corder & Saldanha, 2006). The intensive exploitation of native populations of this species is affecting its long-term economic use and threatening the species. Planting Jussara palm, on the other hand, has some drawbacks, e.g., the long period (8 to 12 years) until the palm heart is ready for harvesting (Morsbach et al., 1998).

Alternatively, *Archontophoenix alexandrae* (F. Muell.) H. Wendl. & Drude, (King palm) is being grown to replace *E. edulis* for the palm heart production. This species also has only one stem, grows at a rate of 1.0 m/year (Lorenzi et al., 2004) and the palm heart is harvestable after 3 years if grown under appropriate conditions (Bovi et al., 2001). This species has been widely used in southern Brazil for palm heart production, especially in the coastal region of Santa Catarina, where *E.edulis* is considered a threatened species (Lorenzi et al., 2004).

Considering their economical and ecological importance, *Archontophoenix alexandrae* and *Euterpe edulis* could be introduced in Agroforestry Systems (AFS). In AFS, forest tree species are combined with crops, animal husbandry or with both, imitating a forest system with a diversity of plant species occupying distinct heights in the canopy (Anderson et al., 1991; Daniel et al., 1999). AFS represent an alternative model of sustainable agriculture by generating additional profits for small producers, maintaining soil fertility, contributing to biodiversity conservation (Anderson et al., 1991), and with a high potential for carbon (C) sequestration and greenhouse gas mitigation (Mutuo, 2005). The increase in above- and belowground biomass in planted forests is high in the first 10 years (Brown et al., 1989), as similarly measured in AFS in the first years after implementation (Santos et al., 2004). Soto-Pinto et al. (2010) estimated an average C accumulation of 95 Mg ha^{-1} in AFS (range of 12 - 228 Mg ha^{-1}).

The species planted in AFS are associated with arbuscular mycorrhizal fungi (AMF - phylum Glomeromycota). In nature, AMF are associated with 90 % of all plant species (Wang & Qiu, 2006) including trees, shrubs, herbs, and crop plants. The uptake of non-mobile nutrients such as P is the main benefit to plants promoted by the fungi, resulting in higher growth rates and improved nutrition; on the other hand, fungi receive carbohydrates translocated from above-ground parts to roots for their growth and sporulation (Smith & Read, 2008). Mycorrhization of plant seedlings generally results in a faster development, higher yields and greater tolerance to environmental stresses at field transplanting (Douds et al., 2007; Hu et al., 2009; Higo et al., 2010; Pellegrino et al., 2011), but this microbial resource has rarely been tested for tropical palm species.

Arbuscular mycorrhizal association is also important to improve growth and nutrition of palm species. In AMF-colonized seedlings of *Bactris gasipaes* (pupunha) significant increases were observed in relation to non-mycorrhizal plants in terms of height at field transplanting stem diameter (22 %), shoot dry biomass (140 %), root dry biomass (89 %), and nutrient uptake (Sudo et al., 1996). Mycorrhizal association increased P and K nutrition of *Elaeis guineensis* (Carvalho, 1997), and plant growth and nutrition of *Euterpe oleracea* (Chu, 1999). Ramos-Zapata et al. (2006) observed that plants of *Desmoncus orthacanthos* associated with AMF had higher survival rates, leaf area and P uptake. Jaiti et al. (2007) showed that AMF positively influenced leaf number, height and biomass of *Phoenix dactylifera*.

The interaction of AMF with *Archontophoenix alexandrae* and *Euterpe edulis* was evaluated herein under greenhouse and field conditions. The aim of this study was twofold: 1) to determine the influence of AMF inoculation on soluble carbohydrate and starch accumulation and 2) to evaluate the effect of mycorrhizal inoculation on plant growth after two years under field conditions. The hypothesis was tested that mycorrhizal association improved allocation of soluble carbohydrates and starch to the roots, and that the inoculation of palm seedlings in nurseries enhances growth under field conditions.

MATERIAL AND METHODS

Study 1: Influence of AMF on growth, soluble carbohydrates and starch under greenhouse conditions

Seeds of *Archontophoenix alexandrae* and *Euterpe edulis* were obtained from the germplasm bank of an experimental station of the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), in Itajaí, Santa Catarina. Seeds were scattered on plastic trays with carbonized rice husk for germination. Three months after germination, seedlings were transplanted to plastic cones (270 mL) filled with sterilized sand (autoclave at 121 ^{o}C for 1 h) and treated with: a) mycorrhizal fungi (AMF) inoculation, and b) control without inoculation (CTL). Soil inoculum containing hyphae, spores, and fragments of roots and substrate was added (10 g per cone) in a furrow before seedling transplanting. The mycorrhizal inoculum consisted of a mixture of the AMF *Acaulospora koskei* SCT400A, *Scutellospora heterogama* PNB102A, *Gigaspora albida* PRN201A and trap culture SCT713 containing mainly *Rhizophagus clarum*. The isolates and trap cultures were obtained from the International Culture Collection of Glomeromycota (CICG) at the Universidade Regional de Blumenau (FURB), in Blumenau, SC - Brazil. The plants were maintained in a greenhouse and watered as needed. Three months after inoculation, plants were fertilized with 10 mL of Long-Ashton nutrient solution containing 10 % of the original P concentration. Each cone was considered a replicate and treatments were arranged in a completely randomized design.

After five months, 20 plants of each treatment were randomly selected to measure biomass production and mycorrhizal colonization (n = 10) and determine chlorophyll, soluble carbohydrates and starch (n = 10). Shoots were excised from the roots, oven-dried at 65 ^{o}C for 72 h, and weighted to obtain shoot dry biomass. Shoots were ground and sent to a State Laboratory (EPAGRI, Caçador, SC) for P determination by spectrophotometry at 410 nm (Sarruge & Haag, 1974).

Roots were stained according to the methodology of Koske & Gemma (1989), with modifications. These modifications were necessary to obtain satisfactory results during staining as original procedures of the methodology resulted in dark roots that impaired appropriate visualization of fungal structures. Roots

were immersed in a 10 % KOH solution for 72 h (solution exchange every 24 h). Thereafter, roots in KOH were placed in a water bath (90 ºC) for 60 min. Roots were washed under tap water and immersed in an alkaline solution of H_2O_2 for 30 min. After washing, the roots were placed in a 1 % HCl solution for 20 min. After HCl removal, the roots were covered by a 0.05 % trypan blue solution and left for another 60 min in a water bath (90 ºC). Roots were maintained in distilled water at 4 ºC until analysis. To verify root colonization, 20 1-cm pieces of fine roots were placed on a slide with PVLG (polyvinyl-lacto-glycerol) and covered with a coverslip. The fungal structures (hypha, arbuscule, vesicles) on each root piece were evaluated under a microscope (Zeiss Axiostar Plus) at 100× magnification. AMF spores were extracted from 50 mL of substrate by wet sieving followed by sucrose centrifugation (20 and 60 %) and counted under a dissecting microscope (Zeiss, Stemi 2000 C).

The chlorophyll content was measured using *ClorofiLOG* (FALKER) three times per plant and averaged. To determine soluble carbohydrates and starch, roots and shoots of entire seedlings were weighed and ground with mortar and pestle and liquid nitrogen. Samples were subjected to a triple extraction by boiling them in 80 % ethanol solution for 5 min. Extracts were then centrifuged three times at 3,000 ×g for 10 min, filtered through a glass microfiber filter and the filtrate solution completed to 10 mL using 80 % ethanol solution. The solid residues were maintained at -20 ºC for starch determination; at the moment of extraction, 1 mL of cold distilled and deionized water and 1.3 mL of 52 % percloric acid were added and maintained at room temperature for 15 min (solution was occasionally stirred with a glass rod). Then, 2 mL of deionized water was added followed by shaking and then centrifugation at 1,500×g for 15 min. The supernatant was filtered once through a glass microfiber filter into a graduated cylinder. To the solid residue was added 0.5 mL of cold deionized and distilled water and 0.65 mL of 52 % percloric acid and centrifuged for a second time at 1,500×g for 15 min. The supernatant was filtered again through a glass microfiber filter and combined with the solution of the first centrifugation. The volume of the filtered supernatant was raised to 10 mL with distilled water. Total soluble carbohydrates and starch were quantified by colorimetric analyses using the phenol sulphuric method of Dubois et al. (1956) by a spectrophotometer at 490 nm absorbance.

Study 2: Field inoculation of *Euterpe edulis* and *Archontophoenix alexandrae*

Seed origin and germination conditions were as described in study 1. Germinated seedlings were transplanted to 270 mL cones and the following treatments applied: 1) inoculation with a mixture of *Acaulospora koskei* SCT400A and *Gigaspora decipiens* PRN107B (AMF), and 2) control without inoculation (CTL). Inoculum of both AMF, obtained from the CICG at FURB, Blumenau, SC, was produced in pot cultures with a sand:expanded clay mixture (1:1), using *Brachiaria brizantha* as host plant.

In June 2008 (160 days after inoculation), inoculated and non-inoculated seedlings were transplanted to an experimental field of EPAGRI, in Itajaí, SC. The seedlings of *Euterpe edulis* were distributed in 3 rows with 36 plants each and those of *Archontophoenix alexandrae* in 6 rows with 18 plants each, at a distance of 1.5 m between the plantlets. Within and among rows, treatments were arranged in a completely randomized design, with 54 replicates per treatment. In natural forests, *Euterpe edulis* grows in the subcanopy and is intolerant to direct light; these seedlings were therefore transplanted to an area shaded by other tree species to mimic environmental conditions this species occurs in naturally.

Height and stem diameter were measured at transplanting (month 0) and 12 and 24 months later using a metric scale. The stem diameter was measured with a digital caliper at *ca.* 3 cm from the soil.

After 24 months of field growth, five plants per treatment were randomly selected and harvested to measure total biomass and to generate allometric equations to predict the plant biomass based on height and stem diameter. Plants were uprooted from the soil using a shovel and a broad-bladed mattock and separated into leaves, stem, and roots. Roots were immersed in water to free roots from attached soil. Leaves, stem, and roots were oven-dried at 60 ºC for 3 days and weighed separately to obtain the dry biomass of each part. Leaves were dried for one week and stem and roots for two weeks to reach constant weight. Plants were sent to the EPAGRI laboratory (Caçador, SC) to quantify carbon. Plant parts were ground and 0.05 g were digested with 10 mL of 0.5 mol/L $K_2Cr_2O_7$ and 20 mL of H_2SO_4 and maintained at room temperature for 30 min. After that, distilled water (200 mL) and 3 - 5 drops of orto-phenantroline were added. Titration was carried out with 0.16 mol/L $FeSO_4.7H_2O$ and results used to calculate C concentrations (expressed in g kg^{-1}).

Statistical analysis

All data were checked for normality of variance using Levene's test and transformed if necessary. Treatments CTL and AMF were compared within each plant species using Student's *t* test ($p < 0.05$) for the greenhouse study. Regression analysis was used to obtain an equation to estimate biomass from height and diameter measurements. Statistical analyses were performed using JMP® (SAS, 2002).

RESULTS

Study 1: Greenhouse conditions

Five months after inoculation, total chlorophyll, chlorophyll *a* and *b* tended to be slightly higher in

AMF plants than in CTL plants for both palm species, but no statistical differences were detected between AMF and CTL treatments (Table 1). No significant difference was detected in shoot dry biomass for both species after 5 months and AMF-inoculated *A. alexandrae* plants had higher P content compared to CTL plants (Table 1).

After 5 months, starch was significantly higher in roots of AMF plants of both palm species compared to CTL plants, but no differences were detected between treatments in shoots (Figure 1a). Soluble sugar of *A. alexandrae* AMF plants was significantly higher in both roots and shoots than in CTL plants. For *Euterpe edulis,* soluble sugars were significantly higher in roots of AMF than of CTL plants (Figure 1b). Only sparse evidence of arbuscular mycorrhizal colonization was observed in *A. alexandrae* and *E. edulis* roots after 5 months, but newly produced spores were detected and averaged 100 spores/50 mL of substrate. No evidence of colonization was detected in CTL plants.

Study 2: Field conditions

At the time seedlings were field-transplanted (June 2008), the height of mycorrhizal *Archontophoenix alexandrae* plants was 16.63 cm and significantly different from CTL plants. No differences in height were observed at this time for *Euterpe edulis* plants (Table 2). After 12 (June 2009) and 24 months (June 2010) under field conditions, no mycorrhizal effect was observed on height and stem diameter for either plant species (Table 2). After 24 months under field conditions, total dry biomass of *A. alexandrae* and *E. edulis* averaged 476.4 g and 174.35 g, respectively, which represented an increase of 32-48 % over the CTL plants (Figure 2). Mycorrhizal plants of *A. alexandrae* and *E. edulis* produced more biomass of leaves, stems and roots than non-mycorrhizal plants (Figure 3). AMF inoculation resulted in an increase of 44 and 65 % in stem biomass for *A. alexandrae* and *E. edulis,* respectively. CTL plants of *A. alexandrae* allocated 47.8 % of their biomass to leaves while in AMF plants resource allocation was more evenly distributed among leaves (39.8 %) and stems (41.2 %) (Figure 3a). Resource allocation in *E. edulis* was similar, regardless of the treatments: 44 % to leaves, 31 - 34 % to stem, and 21 - 24 % to roots (Figure 3b). The total C content was higher in AMF plants of *A. alexandrae* (363.86 g kg^{-1}) than in CTL plants (342.00 g kg^{-1}), but was similar between treatments in *E. edulis* plants (Table 3).

For 24-month-old plants, allometric equations were generated to estimate total and above-ground

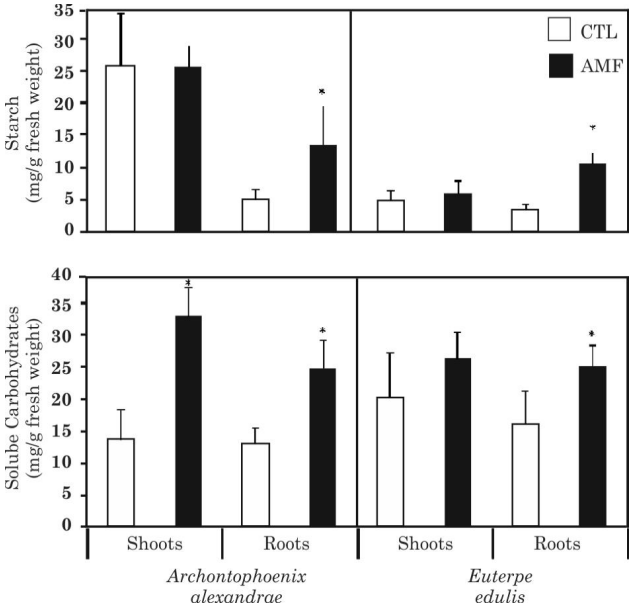

Figure 1. Mean values (± SD) of starch (A) and soluble carbohydrates (B) in roots and shoots of *Archontophoenix alexandrae* and *Euterpe edulis* inoculated with arbuscular mycorrhizal fungi (AMF - black bars) or non-inoculated control (CTL - white bars) after 5 months in a greenhouse. Differences between AMF and CTL within the same plant organ are indicated by an asterisk (*) according to paired *t* test ($p < 0.05$).

Table 1. Chlorophyll, shoot biomass and shoot phosphorus of non-mycorrhizal (CTL) and mycorrhizal (AMF) plants of *Archontophoenix alexandrae* and *Euterpe edulis*, after 5 months in a greenhouse. Values are mean ± standard deviation

Parameter	*Archontophoenix alexandrae*		*Euterpe edulis*	
	CTL	AMF	CTL	AMF
Total Chlorophyll (mg/g fresh biomass)	23.71 ± 5.31	26.10 ± 3.44	27.70 ± 3.26	28.62 ± 4.07
Chlorophyll *a* (mg/g fresh biomass)	19.80 ± 4.32	21.50 ± 2.86	22.81 ± 2.77	23.67 ± 3.45
Chlorophyll *b* (mg/g fresh biomass)	3.91 ± 1.13	4.59 ± 0.64	4.88 ± 0.82	4.95 ± 0.85
Shoot biomass (g)	0.28 ± 0.07	0.24 ± 0.06	0.46 ± 0.09	0.41 ± 0.06
Shoot phosphorus (g/kg)	1.51 ± 0.62	2.19 ± 0.67 *	1.99 ± 0.49	2.30 ± 0.46

* Significant difference ($p<0.05$) between CTL and AMF.

palm biomass based on height and stem diameter (Table 4, Figure 4).

DISCUSSION

This paper provides evidence that AMF inoculation of seedlings of two tropical palm species increases soluble carbohydrate allocation to roots and improves biomass after two years of field growth. Therefore, arbuscular mycorrhizal association does confer an advantage to the establishment of *A. alexandrae* and *E. edulis* in P-poor tropical soils. To our knowledge, this is the first report on the interaction between AMF and *A. alexandrae* and *E. edulis.*

Under greenhouse conditions, AMF increased shoot P only in *A. alexandrae* and no effect was observed on shoot biomass. The absence of a mycorrhizal effect on growth after five months in both species can be explained by the large seed size, as also observed for other tropical tree species (Zangaro et al., 2000; Siqueira & Saggin Junior, 2001; Pasqualini et al., 2007). Palm species have large seeds with high reserves in the endosperm important at the beginning of germination and to provide the necessary nutrients until seedling establishment (Meerow, 1991). Chu (1999) observed that the mycorrhizal effect on *E. oleracea* shoot biomass differed after nine months, according to the inoculated fungal isolate and Ramos-Zapata et al. (2009) observed that mycorrhizal inoculation increased shoot biomass but not the relative growth rate of *Desmoncus orthacanthos* after four months.

Mycorrhizal inoculation had no effect on chlorophyll concentration but increased soluble carbohydrates and starch allocation to plant roots. Low P levels in the growth substrate (Thomson et al., 1990; Grimoldi et al., 2005) associated with a large C sink in mycorrhizal plants leading to increase in photosynthesis rate (Pearson & Jakobsen, 1993) were possibly useful to increase root and shoot soluble carbohydrates. Contrary to our results, Grimoldi et al. (2005) found no significant effect of AMF on starch and soluble carbohydrate in *Lolium perenne*. In arbuscular mycorrhizal symbiosis, 5-20 % of plant photosynthates are allocated to roots to provide substrate for fungal growth (Douds et al., 1988; Jakobsen & Rosendahl, 1990). Part of these carbohydrates flow into the soil C pool by the extensive

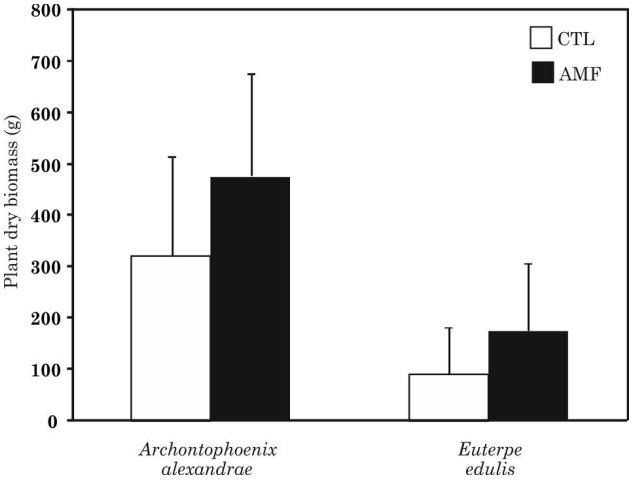

Figure 2. Mean values (± SD, n=5) of total plant dry biomass of *Archontophoenix alexandrae* and *Euterpe edulis* inoculated with arbuscular mycorrhizal fungi (AMF - black bars) or non-inoculated control (CTL - white bars) after 24 months under field conditions.

Table 2. Height and stem diameter of non-mycorrhizal (CTL) and mycorrhizal (AMF) plants of *Archontophoenix alexandrae* and *Euterpe edulis* at transplanting (0 months) and after 12 and 24 months of field growth

Time	*Archontophoenix alexandrae*		*Euterpe edulis*	
	CTL	AMF	CTL	AMF
month		Height (cm)		
0	17.75 ± 1.63 a	16.63 ± 1.83 b	15.70 ± 2.18 a	15.52 ± 2.00 a
12	55.68 ± 11.50 a	51.78 ± 11.17 a	40.66 ± 14.00 a	40.13 ± 11.77 a
24	162.65 ± 29.75 a	157.07 ± 36.14 a	101.16 ± 32.13 a	105.89 ± 37.12 a
		Stem diameter (mm)		
0	3.52 ± 0.52 a	3.60 ± 0.78 a	3.84 ± 0.55 a	3.71 ± 0.47 a
12	18.94 ± 5.05 a	17.59 ± 4.35 a	11.10 ± 2.39 a	10.87 ± 2.75 a
24	68.49 ± 15.13 a	63.56 ± 16.35 a	24.92 ± 7.97 a	26.74 ± 8.85 a

For each palm species, means (± standard deviation) followed by the same letter indicate no statistical difference between CTL and AMF treatments.

hyphal network produced by AMF (Staddon, 1998), and glomalin production, which accounts for 4-5 % of total C and N in some soils (Rillig et al., 2001). Although our results were obtained under greenhouse conditions, if they were extrapolated to field conditions, the higher sink for C in mycorrhizal plants indicates that palm plantations in agroforestry systems (AFS) would contribute significantly to increase soil C sequestration. Indeed, C accumulation of above- and below-ground plant organs (Table 3) under field conditions, especially for *A. alexandrae*, also demonstrates the potential of mycorrhizal plants to contribute to C fixation in AFS. Our results corroborate the suggestion that an appropriate management increases AFS potential for C sequestration and to mitigate the emission of greenhouse gases (Mutuo et al., 2005).

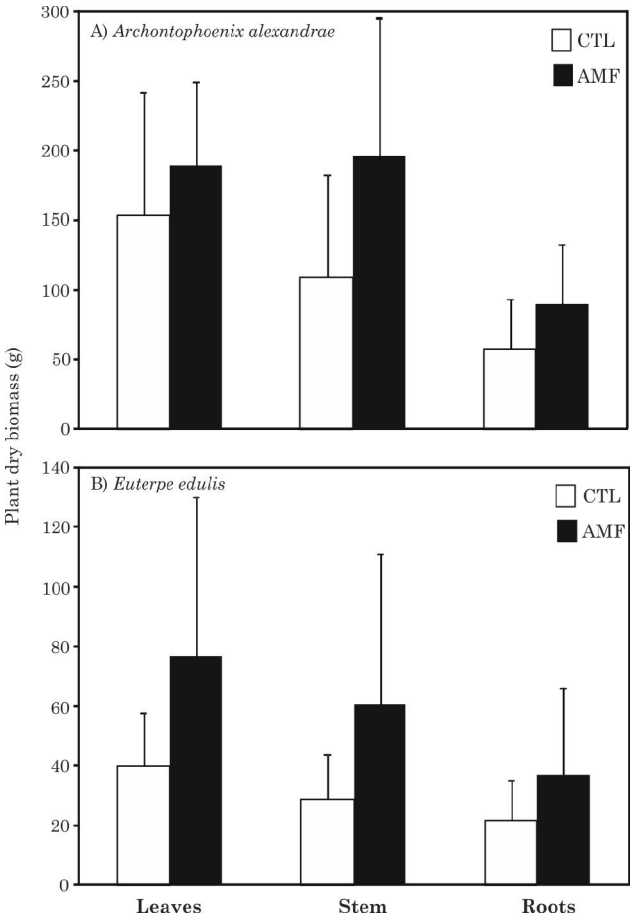

Figure 3. Mean values (± SD, n=5) of leaves, stem, and root dry biomass of (A) *Archontophoenix alexandrae* and (B) *Euterpe edulis* inoculated with arbuscular mycorrhizal fungi (AMF - black bars) or non-inoculated control (CTL - white bars) after 24 months under field conditions.

Seedlings of both palm species showed sparse evidence of mycorrhizal colonization although newly produced spores were recovered from the growth substrate. After five months, sporulation of the AMF species in the original inoculum was detected (100 spores/50 mL of substrate), indicating root colonization. Zangaro et al. (2000) observed low mycorrhizal colonization of *E. edulis* under field conditions and Fisher & Jayachandran (2005) observed cortical AMF hyphae, vesicles, and arbuscules in five palm species. Root colonization of palm species reported in the literature ranges from 13 to 53 % in *Bactris gasipaes* (Sudo et al., 1996; Silva Junior & Cardoso, 2006), 4 to 33 % in *Desmoncus orthacanthos* (Ramos-Zapata et al. 2006), 27 to 86 % in *Phoenix dactylifera* (Jaiti et al., 2007), 4 to 4.2% in *Astrocaryum mexicanum* (Núñez-Castillo & Álvarez-Sánchez, 2003), and 1.5 to 8 % in *Phoenix canariensis* (Morte & Honrubia, 2002). No conclusive explanation was found for the sparse colonization of both palm species studied. Our modifications of the staining protocol proposed by Koske & Gemma (1989) improved the staining quality of palm roots. Root anatomical features of *A. alexandrae* and *E. edulis* are evoked as one factor limiting mycorrhizal colonization. In four palm species, Dreyer et al. (2010) observed the presence of a continuous sclerenchymatic ring in the outer and aerenchyma in the inner root cortex, both anatomical indicators of resistance to mycorrhizal colonization. Fischer & Jayachandran (1999) observed that palm roots are slow-growing and protected by massive

Table 3. Carbon accumulation in *Archontophoenix alexandrae* and *Euterpe edulis* in CTL and AMF treatments after 24 months of field growth

	Archontophoenix alexandrae		*Euterpe edulis*	
	CTL	AMF	CTL	AMF
	Carbon (g kg^{-1})			
Leaves	335.20 ± 52.59	376.80 ± 21.30	321.60 ± 60.32	333.80 ± 50.88
Stem	412.40 ± 25.86	391.60 ± 36.29	388.40 ± 57.75	332.60 ± 47.25
Roots	278.40 ± 39.18	323.20 ± 54.16	313.20 ± 13.75	309.40 ± 41.50
Total	342.00 ± 13.61	363.86 ± 15.83	341.06 ± 31.96	325.26 ± 26.05

Values are mean ± standard deviation.

hypodermal layers. By microscopic observation of root colonization it was possible to detect thick-walled, sclerenchyma-like cells around both palm roots, which possibly acted as a barrier to mycorrhizal colonization. A second factor limiting mycorrhizal colonization could possibly be the large seed size of palm species (Zangaro et al., 2003), which could provide nutrients to early palm growth without the need to become associated with AMF.

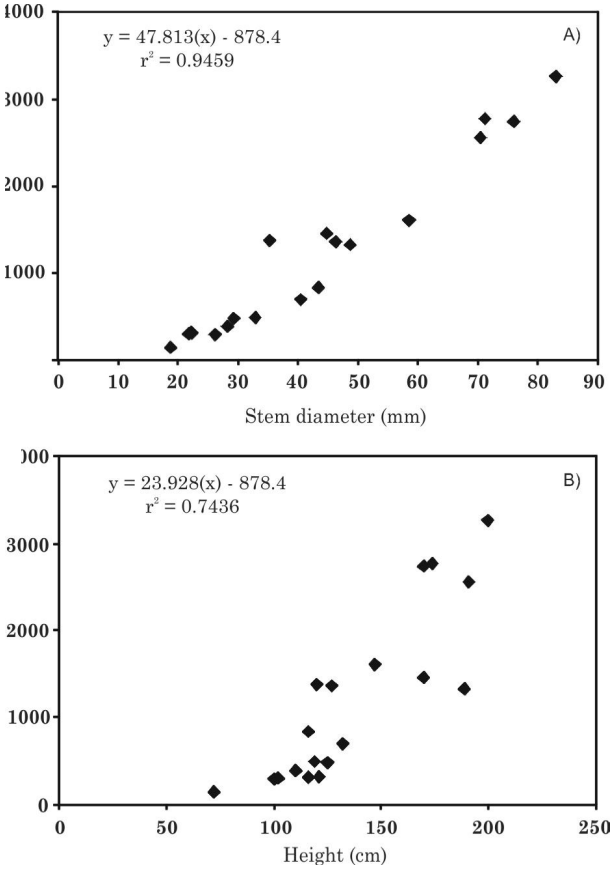

Figure 4. Correlation between plant fresh biomass of *Archontophoenix alexandrae* and *Euterpe edulis* and (A) stem diameter and (B) height after 24 months under field conditions. Correlation coefficients (r^2) are significant at the $p < 0.05$ level.

Inoculation of AMF had a positive effect on biomass production of both palm species under field conditions. This result agrees with previous studies on palm species response to mycorrhizal fungi under greenhouse conditions (Clement & Habte, 1995; Chu 1999; Ramos-Zapata et al., 2009). Long-term experiments with palm species are rare in the literature. Janos (1977) observed a survival rate of 77 % of inoculated *Bactris gasipaes* compared to 33 % for non-inoculated. Under greenhouse conditions, plant height and dry weight of *Phoenix canariensis* inoculated with *Glomus deserticola* and *G. intraradices* were not different from the uninoculated control (Morte & Honrubia, 2002). In the same study, plants inoculated with *G. mosseae* differed significantly from the uninoculated control for the same parameters and all three fungal isolates improved stem diameter. Under field conditions, mycorrhizal inoculation increased leaf area and leaf P content but not height of *Desmoncus orthacanthus,* except when seedlings were introduced into a mature forest (Ramos-Zapata et al., 2006). Results of our study demonstrate for the first time that inoculation of palm seedlings with AMF improves biomass accumulation after 24 months in the field (study 2) and even in the absence of growth promoters under greenhouse conditions (study 1).

Carbon allocation patterns to different above-ground plant organs were also influenced by mycorrhizal inoculation under field conditions. This was more evidenced in *A. alexandrae* than in *E. edulis,* the former distributing the structural C to leaves and stem more evenly when inoculated with AMF than CTL plants. These results have economic implications, since AMF-inoculated plantations of *A. alexandrae* seem to enhance resource allocation to the stem, the commercially explored plant part containing the palm heart. It has been demonstrated that resource allocation to plant organs are altered by co-occurring AMF isolates (Streitwolf-Engel et al., 1997; van der Heijden et al., 2003). Further studies with both palm species should verify the effect of inoculation to screen for possible co-occurring AMF isolates that promote resource allocation to the stem.

This study demonstrated that AMF inoculation of *A. alexandrae* and *E. edulis* can increase plant biomass

Table 4. Allometric equations generated from 24-month-old field plants to estimate total and above-ground biomass of *Archontophoenix alexandrae* and *Euterpe edulis*

Biomass	Allometric equation	r^2	p
Total	$\hat{y}$ = -2086.69 + 23.928019*(H)	0.74	< 0.0001
	$\hat{y}$ = -878.3965 + 47.81341*(D)	0.95	< 0.0001
Above-ground	$\hat{y}$ = -1634.663 + 18.79031*(H)	0.72	< 0.0001
	$\hat{y}$ = -702.1219 + 37.924452*(D)	0.93	< 0.0001

$\hat{y}$ = biomass, H = height in cm, D = diameter in mm.

after 24 months in the field, suggesting that mycorrhizal inoculation is a feasible biotechnological strategy for palm seedling production in nurseries. This method is an approach to increase stem biomass during the establishment of commercial plantations of *A. alexandrae*. For *E. edulis*, a palm species considered threatened due to illegal overexploitation, inoculation should be part of reintroduction programs of this species into secondary forests to increase its abundance. Microbial inoculants including AMF as tested in this study are important resources in tropical soils to improve plant biomass accumulation and reduce fertilizer application.

CONCLUSIONS

1. Mycorrhizal colonization of *A. alexandrae* and *E. edulis* by arbuscular mycorrhizal fungi has the potential to increase allocation of carbohydrates to roots, representing a pathway for underground carbon fixation.

2. Inoculation of palm species with arbuscular mycorrhizal fungi under nursery conditions is an important strategy to increase plant growth and biomass production after field transplanting.

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