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Increase in biomass of two woody species from a seasonal dry tropical forest in association with AMF with different phosphorus levels

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ABSTRACT

The study aimed at assessing whether there is association between arbuscular mycorrhizal fungi (AMF) and two woody species from a seasonal dry tropical forest, *Poincianella pyramidalis* and *Cnidoscolus quercifolius*, besides the occurrence of an increase in biomass under different availability of phosphorus (P) in the soil. The experimental design was completely randomized, with a factorial design with two mycorrhizal levels [inoculated (+AMF) and control (−AMF)] × six phosphorus levels (3, 9, 15, 21, 27, 33 mg dm^{-3}). The presence of mycorrhizal structures, mycorrhizal growth, mycorrhizal efficiency, leaf relative water content (RWC) and gas exchange were evaluated. +AMF plants of both species had mycorrhizal structures, while −AMF showed no structures. In *P. pyramidalis*, +AMF plants had benefits with increasing phosphorus level, while −AMF plants had increases biomass only at the P9 level if compared to the P3-AMF level. The total leaf area was correlated with total dry weight (TDW) in +AMF and −AMF plants. However, +AMF plants had more responses in RWC. In *C. quercifolius*, +AMF and −AMF plants did not differ in RWC and showed reductions in gas exchange with increased phosphorus level. However, these reductions were lower in +AMF plants, besides having a better performance over −AMF plants. The growth and mycorrhizal efficiency were higher at the P15 level, and relationship between total leaf area and TDW were significant only in +AMF plants. Thus, both species perform association with AMF and show increases in growth. The concentrations of phosphorus in the soil for *P. pyramidalis* (33 mg dm^{-3}) and *C. quercifolius* (15 mg dm^{-3}) are indicated for increased effectiveness of mycorrhization, promote increases in gas exchange and growth in +AMF plants.

1. Introduction

Under natural conditions plants grow in association with various soil microorganisms, besides the intrinsic adjustment mechanisms (Ruiz-Lozano et al., 2012) considered as important strategies to withstand adverse conditions (Pagano et al., 2011). AMF may account for almost 50% of the microbial biomass in tropical ecosystems (Olsson et al., 1999), establishing a symbiotic association with the roots of 80–90% of terrestrial plants (Gadkar et al., 2001; Smith and Read, 2008; Van der Heijden et al., 1998), including angiosperms, gymnosperms, pteridophytes and briophytes (Smith and Read, 2008). Mycorrhizal association was recorded from the first land plants. Among the benefits provided to the host, the nutritional status improvement, especially in the capture of nutrients with low mobility in soil such as P; the increased biomass, water absorption through aquaporins, soil structuring, higher CO_2 assimilation rate, increased growth and increased water use efficiency can be emphasized (Bolandnazar et al., 2007; Drüge and Schönbeck, 1992; Neumann et al., 2009; Querejeta et al., 2007; Ruiz-Lozano and Aroca, 2010; Smith and Read, 2008).

A critical criterion in the association between plant and AMF is the availability of nutrients in the soil (Zangaro et al., 2000). Root colonization usually occurs in soils with low availability of nutrients (Rillig, 2004) and varies among host and AMF species, since establishing a symbiotic relationship requires a fine adjustment (Hause et al., 2007). The presence of mycorrhizal colonization in plants, associated with the increased growth promoted by inoculation, indicates the degree of mycotrophy. It can be high or low (Siqueira and Saggin-Júnior, 2001) and such responses may vary. For the tree species, benefits provided by the mycorrhizal colonization on growth and nutrition occur in the

Abbreviations: *A*, net photosynthetic rate; *E*, transpiration rate; AMF, arbuscular mycorrhizal fungi; g_s, stomatal conductance; P, phosphorus; PPFD, photosynthetic photon flux density; RDW, root dry weight; RWC, relative water content; SDW, shoot dry weight; TDW, total dry weight; VPD, vapor pressure deficit; WUE, water use efficiency.

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restricted limits of P content (Anjos et al., 2005; Costa et al., 2005; Oliveira et al., 2015; Samarão et al., 2011; Sena et al. 2004).

The nutrient availability is limited in seasonal dry tropical forests, encouraging mycorrhizal establishment. It can be favored by adding phosphorus, which promotes increases in the total number of hyphae (Bago et al., 2000; Nielsen et al., 2002). The association of plants with microorganisms has been identified as important survival strategy in semiarid environments (Pagano et al., 2011). However, little is known about the contribution of mycorrhizal mutualistic relationship in the development of native species in these regions (Maia et al., 2010).

In this scenario, the present study aims at evaluating the occurrence of association with AMF in two native, endemic and widespread woody species *Poincianella pyramidalis* and *Cnidoscolus quercifolius* from a seasonal tropical dry forest in Brazil (Maia, 2004). For such, the following questions needed to be answered: (1) Do *P. pyramidalis* and *C. quercifolius* associate with AMF? (2) Are both species under study physiologically favored by the increased availability of phosphorus nutrients? (3) Are both species favored by the mycorrhizal association? Therefore, inoculated and non-inoculated seedlings were exposed to different levels of phosphorus under greenhouse conditions.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted in a greenhouse (8°08′58″S; 34°56′55″W), with a mean temperature of 30 °C and a relative air humidity of 60%. The plants were kept under pot capacity (300 mL). The design of the study was completely randomized, with a factorial design with two mycorrhizal levels [inoculated (+AMF) and control (−AMF)] × six phosphorus levels (3, 9, 15, 21, 27, 33 mg dm^{-3}). Each treatment had six replicates, totaling 72 experimental units. These concentration intervals were defined from the results reported in the literature, with positive responses from the AMF for the growth of tree species with lower levels of phosphorus (Costa et al., 2005; Oliveira et al., 2015).

The soil used in the experiment was collected in northeastern of Brazil (07° 33′ 38″ S; 35° 00′ 09″ W), due to its low phosphorus content (P: 3 mg/dm^3; pH: 5.7 H_2O; Ca: 0.65 $cmol_c/dm^3$; Mg: 0.6 $cmol_c/dm^3$; Na: 0.04 $cmol_c/dm^3$; K: 0.07 $cmol_c/dm^3$; Al: 0.55 $cmol_c/dm^3$; H: 1.75 $cmol_c/dm^3$; S: 1.4 $cmol_c/dm^3$; CEC: 3.7 $cmol_c/dm^3$). It is classified as Yellow Podzolic. The soil was entirely sterilized by autoclaving at 121 °C for 2 h in two alternate days, and dried in a forced ventilation oven at 70 °C for 24 h.

AMF isolates [*Acaulospora longula* Spain & NC Schenck (URM AMF 07), and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler (URM AMF 03)] were provided by the Inoculum Bank of the Laboratory of Mycorrhizas at the Department of Mycology of Federal University of Pernambuco. Isolates of both AMFs were placed together in pots with 5 kg of autoclaved soil with *Panicum miliaceum* L. and *Sorghum bicolor* (L.) Moench in pot cultures to increase spore density for three month. The number of spores was determined by wet sieving in 50 g of soil, followed by centrifugation at 700 x *g* for 3 min in sucrose (40% w/v) (Jenkins, 1964). The quantification of the spores was performed with a stereoscopic microscope with a 40x magnification on plates with concentric channels (Gerdemann and Nicolson, 1963).

The seeds of *P. pyramidalis* (Tul.) LP Queiroz and *C. quercifolius* Pohl were provided by the Reference Center for the Recovery of Degraded Areas (CRAD)—UNIVASF/Petrolina-PE. The seeds were sterilized in 1% hypochlorite (v/v) for 5 min, rinsed in deionized water and placed to germinate in trays containing sterilized washed sand. After 20 days, the plants were transferred to 100 mL pots with autoclaved soil. The plants destined for inoculation received 150 spores of each AMF at the root region, totaling 300 spores per plant. Control plants received the same amount of inoculum soil without AMF propagules. After 30 days under these conditions, the plants were transferred to pots containing 2 kg of autoclaved soil with its phosphorus level determined by the application of simple superphosphate (P_2O_5), where the first level had no P addition (soil concentration was 3 mg dm^{-3}) followed by five levels with addition: 9, 15, 21, 27, 33 mg dm^{-3}, representing the levels P3 (soil concentration), P9, P15, P21, P27 and P33, respectively. When plants completed four months of development, mycorrhizal structures in roots, biometrics, total leaf area, biomass, leaf relative water content, soil water status and gas exchange were evaluated.

2.2. Mycorrhizal structures

The verification of root colonization was conducted by observing fungal structures. For this, the roots were clarified with KOH (10%, w/v) for 24 h, washed with distilled water and stained with trypan blue in lactoglycerol (0.05%, w/v) for 12 h (Phillips and Hayman, 1970). The evaluation was performed using the glass plate method with 1 cm root fragments from all individuals with a microscope with a 40× magnification (Giovannetti and Mosse, 1980).

2.3. Growth, mycorrhizal increase and mycorrhizal efficiency

The biometric data on the number of leaves, height (cm) and diameter (mm) was taken on the last day of the experiment. The number of leaves was counted, the height was obtained with a millimeter ruler and the diameter was measured with a digital caliper. Subsequently, leaves were scanned and the total leaf area was calculated using the Image Pro software. Afterwards, shoots and roots were placed in a forced ventilation oven at 70 °C for 5 days. The material was weighed on precision scales (AND H200, Tokyo, JP), thus obtaining total dry weight. Using biometrics (number of leaves, height, diameter and total leaf area) and dry biomass data [shoots (SDW), roots (RDW) and total biomass (TDW)], the mycorrhizal increase (100[(X − Y)]/Y) and the mycorrhizal efficiency (100[(X − Y)]/X) were calculated, where X is mycorrhizal plants and Y is control plants (Weber et al., 2004).

2.4. Leaf relative water content (RWC) and soil water status

Leaf disks with a known size were collected at 06:00 and immediately weighed on a precision scale to obtain the fresh weight (FW). Then, the leaf disks were soaked for 24 h in deionized water and reweighed to obtain the swelling weight (SW). Subsequently, the disks were dried in a forced ventilation oven for 48 h and reweighed to obtain the dry weight (DW). The leaf relative water content (RWC) was calculated by the following formula, according to Barrs and Weatherley (1962): RWC (%) = [FW − DW/SW − DW] × 100. Soil moisture was obtained in all replicates used for the evaluation of gas exchange using the meter Falker HFM 2030 (v/v), the soil moisture measured was 12.2% in the pots for the two species.

2.5. Gas exchange

Gas exchange was measured in expanded and non-senescing leaves through an infrared gas analyzer (IRGA, ADC, model LC-Pro; Hoddesdon, UK), obtaining stomatal conductance (g_s), net photosynthetic rate (A) and transpiration rate (E). The water use efficiency (WUE) was calculated by the ratio A/E. Measurements were taken from 09:00 to 10:00 with a 1000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and vapor pressure