KAREN MIRELLA SOUZA MENEZES

CRESCIMENTO INICIAL DE MUDAS DE ESPÉCIES ARBÓREAS NATIVAS PRODUZIDAS EM SUBSTRATOS INOCULADOS COM FUNGOS MICORRÍZICOS ARBUSCULARES

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Doctor Scientiae*.

Orientadora: Maria Catarina Megumi Kasuya

Coorientadores: Haroldo Nogueira de Paiva
Marliane de Cássia S. da Silva

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Á minha mãe Josefa Menezes

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RESUMO

MENEZES, Karen Mirella Souza, D.Sc., Universidade Federal de Viçosa, maio de 2022. **Crescimento inicial de espécies arbóreas nativas inoculadas com fungos micorrízicos arbusculares**. Orientadora: Maria Catarina Megumi Kasuya. Coorientadores: Haroldo Nogueira de Paiva e Marliane de Cássia S. da Silva.

Áreas de florestas nativas tem sofrido danos severos gerando ambientes degradados, visando a recuperação dessas áreas o mercado de produção de mudas de espécies arbóreas nativas vem ganhando espaço. A fim de suprir essa demanda a inoculação de mudas nativas com fungos micorrízicos arbusculares (FMA) pode auxiliar no desenvolvimento das mudas produzidas em viveiro, além de auxiliar no estabelecimento em campo. No entanto, o tipo de substrato utilizado pelos viveiristas restringir o funcionamento dessa simbiose refletindo no desenvolvimento e desempenho das espécies arbóreas em campo. Na primeira etapa da Tese, realizou-se um experimento em delineamento inteiramente casualizado com quatro tratamentos: dois substratos, Antuérpia e Ouro Verde e dois tratamentos de inoculação (sem e com espécies de FMA) com cinco repetições e um segundo experimento avaliando o efeito da inoculação e do substrato sob a assembleia de FMA residente na rizosfera de seis espécies arbóreas nativas utilizando um delineamento experimental semelhante ao primeiro estudo. No primeiro experimento foram avaliados: a altura, o diâmetro do caule, a biomassa seca da parte aérea e a colonização micorrízica. A inoculação micorrízica não promoveu o crescimento inicial das 13 espécies arbóreas nativas estudadas, porém, todas as plantas foram colonizadas pelos FMA. Em geral, o substrato Antuérpia promoveu melhor desenvolvimento e maior colonização micorrízica das mudas em comparação àquelas crescidas no substrato Ouro Verde. No segundo estudo, a assembleia de FMA é influenciada pela adição do inoculante, modificando a estrutura e a composição da comunidade nativa. Além disso, constatou-se que as mudas de espécies arbóreas nativas podem associar-se preferencialmente a certos grupos de FMA, sendo os gêneros *Acaulospora*, *Glomus* e *Paraglomus* os mais abundantes nos substratos estudados. Na segunda etapa da Tese, mudas de seis espécies arbóreas nativas foram transplantadas para o campo. Adotou-se o delineamento em blocos casualizado em parcelas subdivididas, sendo as parcelas compostas pelos substratos (Antuérpia e Ouro Verde), e as subparcelas pelos tempos (6 e 12 meses). Foram avaliadas a altura, o diâmetro do caule, a biomassa seca parte aérea (BSPA), a taxa de crescimento relativo (TCR), taxa de volume do tronco (TVT), a área foliar total (AFT) a área foliar específica (AFE), bem como os atríbutos fisiológicos (fluorescência variável e máxima - Fv/Fm, Clorofila *a*, *b* e total) e o número de glomerosporos. Como

esperado, houve um forte efeito do tempo nos parâmetros de crescimento e na fisiologia da maioria das espécies arbóreas nativas estudadas. Todas as espécies arbóreas, como esperado tiveram maior altura, diâmetro do caule e TVT aos 12 meses. Por outro lado, não foi observado efeito do substrato nos parâmetros avaliados, com exceção de *Tabernaemontana hystrix* que teve sua razão Fv/Fm influenciada pelo substrato. Constatamos que aos seis meses *Pterogyne nitens* e *Joannesia princeps* apresentaram maior AFE, enquanto *Colubrina glandulosa* apresentou maior conteúdo de clorofila *a* e total aos 12 meses (estação chuvosa). Conclui-se que o substrato influencia na eficiência da inoculação micorrízica em fase de viveiro, porém, em campo as estações são os drivers mais importantes a serem considerados no processo de restauração de áreas degradadas.

Palavras-chave: FMA Nativo. Inoculação. Viveiro. Diversidade micorrízica. Substrato. Fluorescência da clorofila

ABSTRACT

MENEZES, Karen Mirella Souza, D.Sc., Universidade Federal de Viçosa, May, 2022. **Initial growth of seedlings of native tree species produced in substrates inoculated with arbuscular mycorrhizal fungi**. Adviser: Maria Catarina Megumi Kasuya. Co-advisers: Haroldo Nogueira de Paiva and Marliane de Cássia S. da Silva.

Areas of native forests have suffered severe damage generating degraded environments, aiming at the recovery of these areas the market for the production of seedlings of native tree species has been gaining ground. In order to meet this demand, the inoculation of native seedlings with arbuscular mycorrhizal fungi (AMF) can help in the development of seedlings produced in the nursery, in addition to assisting in the establishment in the field. However, the type of substrate used by nurseries restrict the functioning of this symbiosis, reflecting on the development and performance of tree species in the field. In the first stage of the Thesis, an experiment was carried out in a completely randomized design with four treatments: two substrates, Antuérpia and Ouro Verde and two inoculation treatments (with and without AMF species) with five replications and a second experiment evaluating the effect of inoculation and substrate under the rhizosphere-resident AMF assemblage of six native tree species using an experimental design similar to the first study. In the first experiment, height, stem diameter, shoot dry biomass and mycorrhizal colonization were evaluated. The mycorrhizal inoculation did not promote the initial growth of the 13 native tree species studied, however, all plants were colonized by AMF. In general, the Antuérpia substrate promoted better development and greater mycorrhizal colonization of the seedlings compared to those grown on the Ouro Verde substrate. In the second study, the AMF assemblage is influenced by the addition of the inoculant, modifying the structure and composition of the native community. Furthermore, it was found that seedlings of native tree species may preferentially associate with certain groups of AMF, with the genera *Acaulospora*, *Glomus* and *Paraglomus* being the most abundant in the substrates studied. In the second stage of the Thesis, seedlings of six native tree species were transplanted to the field. A randomized block design was adopted in subdivided plots, with the plots being composed by the substrates (Antuérpia and Ouro Verde), and the subplots by the times (6 and 12 months). The height, stem diameter, shoot dry biomass (SDB), relative growth rate (RGR), trunk volume rate (TVR), total leaf area (TLA) and specific leaf area (SLA) were evaluated, as well as the physiological attributes (variable and maximum fluorescence - Fv/Fm, Chlorophyll a, b and total) and the number of glomerospores. As expected, there was a strong effect of time on the growth parameters and physiology of most native tree species studied. All

tree species, as expected, had greater height, stem diameter and TVR at 12 months. On the other hand, there was no effect of the substrate on the parameters evaluated, with the exception of *Tabernaemontana hystrix* which had its Fv/Fm ratio influenced by the substrate. We found that at six months *Pterogyne nitens* and *Joannesia princeps* had higher SLA, while *Colubrina glandulosa* had higher chlorophyll a and total chlorophyll a content at 12 months (rainy season). It is concluded that the substrate influences the efficiency of mycorrhizal inoculation in the nursery phase, however, in the field, the seasons are the most important drivers to be considered in the process of restoration of degraded areas.

Keywords: Native FMA. Inoculation. Nursery. mycorrhizal diversity. Substrate. chlorophyll fluorescence

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1. GERAL INTRODUCTION

The global human population is expected to reach 8.5 billion in 2030 (ONU, 2019), and this increasing can impact on the functioning of ecosystem services due to the change in land use (Millenium Ecosystem Assessment, 2005; Constanza et al. 2014). These changes have mainly affected forest environments, which have a fundamental role in nutrient cycling, through carbon absorption, litter production, nutritional input to the atmosphere and export of nutrients from the soil, and also becoming a source of available nutrient (Foster and Bhatti, 2006). In Brazil, to recover forest ecosystem services, seedlings, mainly from native tree species have been recommended. However, for the success of recovering process, the following aspects must be considered (Franco and Faria, 1997): (a) giving preference to fast-growing plants to ensure rapid soil cover and that are adapted to local conditions; (b) use forest species with the potential to form symbiosis with nitrogen-fixing bacteria and with mycorrhizal fungi; (c) give preference to native and pioneer species; (d) choose tree species not only for economic benefits, but also for those with high plasticity, such as resistance to drought, pollution and pathogens; (e) that are able to attract pollinators and have the potential to bioremediate. In addition, choosing forest species that produce litter in abundance and with good nutritional quality, as the type of material deposited in the soil can help restore degraded areas by stimulating soil microbial activity (Finkenbein et al. 2013), since microorganisms are involved in the decomposition of organic matter. Thus, there is a great interest in producing seedlings using technologies that allow plants to become more resistant and resilient under adverse conditions in the field. Thus, the use of symbiotic microorganisms, such as arbuscular mycorrhizal fungi (AMF), added to the cultivation substrate can help in the initial growth of seedlings in the nursery, and consequently in the establishment of seedlings in the field (Souza et al. 2012; Berruti et al. 2016), assisting in the recovery of degraded areas (Asmelash et al. 2016). Mycorrhizal association can promote other benefits such as greater tolerance to water stress (Augé et al. 2015) and ensure better absorption of nutrients such as P (Smith and Smith, 2011). In addition, the hyphae produced by AMF help aggregating soil particles through a glycoprotein called glomalin (Rillig, 2004). However, the success of symbiosis can be affected by the type of cultivation substrate used for seedlings development. Thus, a good substrate for seedling production must have adequate physical, chemical and biological characteristics, as well as low cost, in order to guarantee higher quality and seedling growth (Pascual et al. 2018). The use of substrates with adequate physical and chemical characteristics and inoculated with AMF can produce good quality seedlings (Dalanhol et al. 2016). In addition to providing nutritional support to plants, the

physical characteristics of the substrates can influence the growth and AMF can promote plants growth, since the growth medium is the link between the plant and the microorganisms (Oliveira Júnior et al. 2019; Abaurre et al. 2020). Thus, it is important to consider previous studies evaluating and selecting substrates with the most promising physical and chemical characteristics in favoring and potentiating the role of AMF during in seedling production (Abaurre et al. 2020 and 2021).

During production of seedlings of *Semanea saman* (Jacq.) Merr. in different substrates, it was found that the growth parameters were favored in the growth media that presented greater total porosity, air porosity, water and magnesium availability and was negatively affected by the pH and apparent density of the substrate (Abaurre et al. 2020 and 2021). However, the reintroduction of native forest tree species and the effects of the introduction of symbiotic microorganisms in different substrates on the establishment and physiological responses of plants over time is still poorly studied, since seasonal effects have a strong effect on plant fitness (Afonso et al. 2020), affecting their physiological functions and field performance. Furthermore, some species may have their photosynthetic apparatus compromised due to seasonal fluctuations and the growth substrate (Dos Anjos et al. 2015; Afonso et al. 2020). In this context, study of functional traits, such as morphological and physiological characteristics, are of great relevance to ensure success in plant restoration, mainly in degraded areas.

As reported in the literature, the composition of substrates and the inoculation with AMF propagules can indirectly influence the production of photosynthetic pigments due to the availability of water and nutrients, however, the answers are not clear, especially in the field. In a nursery, production of chlorophyll *a*, *b* and total in *Tabermaemontana catharinensis* seedlings were not influenced by substrate composition on the production of (Afonso et al. 2020). On the other hand, in *Eugenia dysenterica* seedlings it was observed that the presence of organic residues in the substrate composition favors the Fv/Fm ratio, electron transport rate and plant growth (Mota et al. 2016), showing that these physiological parameters are dependent on plant species, which are not very understanding for Brazilian native tree species.

So, in order to expand knowledge about the benefits of the mutualistic association between AMF and native tree species, as well as the type of substrate for seedling production, the hypothesis of the present study was that inoculation with AMF helps the growth and establishment of seedlings of native trees under field conditions, aiming at the revegetation of degraded areas, and that the type of substrate also influences the efficiency of the symbiosis reflected in the development of seedlings.

Therefore, this thesis was divided into three stages with the following objectives:

i) To evaluate seedling growth and mycorrhizal colonization of native tree species of the Brazilian Atlantic Forest produced in two substrates when inoculated with AMF produced by the on-farm method, under commercial nursery conditions.

ii) To investigate if inoculation exotic AMF isolates and the cultivation substrate promote changes in the native AMF assemblage in the rhizosphere of six native tropical tree species and if plant hosts preferentially elect some AMF species.

iii) To evaluate, in the field, the effects of the introduction of seedling cultivated in two types of substrates, one containing AMF propagules (Antuérpia) and another without inoculation (Ouro Verde) in the establishment of seedlings used for revegetation of degraded pastures, as well as to observe the effects of the dry and rainy seasons and the possible changes that these factors can cause on plant physiology and mycorrhizal dynamics in the field.

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3. Chapter 1: Written according to Symbiosis

Native tree species of Atlantic Forest show different seedlings growth in response to AMF inoculation and type of substrate

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Abstract

The impact of arbuscular mycorrhizal fungi (AMF) inoculation and the type of substrate on growth and mycorrhizal symbiosis was studied in seedlings of 13 native pioneer and secondary tree species from the Atlantic Forest. The AMF inoculum used was produced by on farm method by multiplying AMF from an area of Atlantic Forest. The design was completely randomized in a factorial (2 x 2) which consisted of two substrates (Antuérpia and Ouro Verde) and two inoculation treatments (control and inoculated with a mix of AMF species). In general, mycorrhizal inoculation did not bring benefits in the initial growth of both pioneer and secondary species, except for *Albizia niopoides*, which grew better when was inoculated. The Antuérpia substrate provided the best development of seedlings, mainly of pioneer species, as it promoted improvements in at least one of the studied growth parameters. Furthermore, the substrate Antuérpia favored symbiosis with AMF than substrate Ouro Verde, providing the highest percentages of mycorrhizal colonization in both successional groups. The results suggest that the efficiency of mycorrhizal inoculation can be modulated according to the type of substrate and that the most significant response occurred when the seedlings were produced in the Antuérpia substrate, this effect being more evident in the fast-growing species and should be a strategy of management considered by nurseries.

Key words: pioneer species, secondary species, native AMF, inoculation, nursery

Introduction

As The restoration process of degraded areas has been shown to be favored when plant seedlings are introduced together with beneficial soil microorganisms (Prado et al. 2019; Jordão et al. 2021; Zanchi et al. 2021), since these microorganisms improve plant survival and biomass accumulation accelerating the establishment of plant communities (Pascual et al. 2018). Inoculating plant seedlings at the nursery stage with beneficial microorganisms is an important step to suppress diseases, to increase nutrient availability and to induce plant hormones production, resulting in seedlings with better quality to be used in restoration processes (Algnolucci et al. 2020). Among soil microbial groups, arbuscular mycorrhizal fungi (AMF), establishing a mutualistic symbiosis with plant roots, have a key role to improve plant growth and nutrition and to structure plant communities (van der Heijden et al. 1998). However, the success of AMF inoculation in seedlings on nursery conditions depends on previous knowledge of the host ability to establish the symbiosis and the degree of mycorrhizal responsiveness (Siqueira and Saggin-Júnior, 2001) and the substrate chemical and physical characteristics that must be conducive to the establishment of the mycorrhizal symbiosis.

Native woody species from the Brazilian Atlantic Forest, one of the global hot spots of biodiversity, inoculated with AMF have shown improved growth and phosphorus absorption under nursery conditions (Pasqualini et al. 2007; Zangaro et al. 2015; Goetten et al. 2016; Fernandes et al. 2019; Silva et al. 2020). However, plants from early successional groups (pioneer species) had higher mycorrhizal dependence and higher colonization when compared to late secondary species (Zangaro et al. 2000 and 2003). Most woody species have shown positive responses to inoculation, regardless of whether it is during the seedling formation period or before transplanting to the field (Pouyú-Rojas and Siqueira, 2007). Furthermore, Carneiro et al. (1996) when testing the effect of the addition of superphosphate in 31 woody species after inoculation with *Glomus etunicatum* (=*Claroideoglomus etunicatum*) and *Gigaspora margarita* found that the mycorrhizal colonization rate of the studied plant species was greater than 20% as a function of inoculation. However, Koziol and Bever (2016) observed that late successional species are more dependent on mycorrhizal inoculation with greater growth than early succession species, which demonstrate that the success of mycorrhizal inoculation, in addition to the management adopted for seedling production, depends on the successional stage of plants. Although the benefits of AMF inoculation during seedlings production have been demonstrated under nursery conditions, this type of management practice is not commonly adopted among nurseries in Brazil, mainly because commercially AMF-based

inoculants are scarce in the market and relatively expensive. Thus, the production of an AMF-based inoculant using the on-farm method is promising (Douds et al. 2005) and represents an alternative for inoculum production used by nursery owners to inoculate seedlings due to its low cost (Prado et al. 2019) For instance, inoculation of *Rhizophagus clarus* and *Claroideoglomus etunicatum* produced by the on-farm method increased height and stem diameter of native timber species *Luehea divaricata*, *Centrolobium robustum,* and *Cedrela fissilis* and *Schinus terebinthifolius* (Schoen et al. 2016). The on-farm method utilizes grasses species as the host plant to multiply AMF using a variety of soil-based or composted substrates mixed with inert materials (Gaur et al. 2000; Douds et al. 2010).

The chemical, physical and biological properties of the substrate are key factors to be considered during native tree seedlings production (Pascual et al. 2018). A good substrate must have chemical and physical properties, such as pH, nutrient content, cation exchange capacity (CEC), electrical conductivity and C/N ratio, as well as texture, porosity, surface size, particle and water-holding capacity, which promote plant health and rapid growth, together with the presence of beneficial microorganisms such as AMF and growth-promoting rhizobacteria (Pascual et al. 2018; Abaurre et al. 2021). Substrates with adequate chemical and physical properties and inoculated with AMF result in the production of good quality seedlings (Dalanhol et al. 2016). This corroborates with previous studies that evaluated and selected substrates with the most promising physical and chemical characteristics in favoring and potentiating the role of AMF in seedling production (Abaurre et al. 2020 and 2021).

However, the origin and the quality of the AMF inoculum certainly affects the production of seedlings with good quality, especially when it is considered that the substrate used by nurseries are not sterilized and therefore contains other microrganisms that can impact seedling growth (e.g., pathogens) (Matlz and Treseder, 2015). On the other hand, inoculation with exotic AMF on an already established mycorrhizal community can affect the presence of these native microorganisms capable of colonizing and benefiting plants (Phillips et al. 2020), which can lead to partial or complete replacement, in addition to increasing competition with native fungi (Janouskova et al. 2013; Hart et al. 2018). This demonstrates that the origin of the inoculant must be considered when choosing the AMF species to be used in native forest species (Mummey et al. 2009).

In addition, the demand to produce seedlings of native tree species has increased, especially after the failure of the Fundão dam in Mariana-MG, Brazil, in November 2015, which led to a 71% reduction in the native vegetation of the Atlantic Forest that borders the Rio Doce (Pires et al. 2017). To reforest 40.000 hectares of permanent preservation areas (PPA), it will

be necessary to produce 20 million seedlings of native tree species (Renova, 2021. The goal of this work was to evaluate seedling growth and mycorrhizal colonization of native tree species of the Brazilian Atlantic Forest produced in two substrates when inoculated with AMF produced by the on-farm method, under nursery conditions. We tested the hypothesis that the responses to mycorrhizal inoculation differ among native Atlantic Forest tree species as a function of successional, and we also tested hypothesis that the interaction between substrates and AMF inoculation can plant growth parameters), and finally, the physical and chemical characteristics of the substrates can influence the growth of seedlings.

Material and Methods

Experimental conditions

The experiment was carried out from September 2019 to February 2020 (150 days), in a commercial nursery named Ouro Verde, located in the municipality of Belo Oriente, Minas Gerais, Southeast of Brazil (19°11'39.7"S 42°24'50.9"W). The region climate is of the Aw type, according to Koppen's classification, with an annual average temperature of 21.4 ºC. Below shows the 13 native tree species selected and grouped according to their successional group (Table 1).

Table 1. Native tree species of the Atlantic Forest Biome selected for the production of seedlings in two types of substrates without and with inoculation with AMF.

Species	Common name	Family
	Pioneer species	
Albizia niopoides (Spruce ex Benth.) Burkat	Farinha-seca	Fabaceae
Anadenanthera peregrina (L.) Speg.	Angico-vermelho	Fabaceae
Colubrina glandulosa Perkins	Saguaraji	Rhamnaceae
Guazuma ulmifolia Lam.	Mutamba	Malvaceae
Plathymenia reticulata Benth.	Vinhático	Fabaceae
Pterogyne nitens Tul.	Amendoim-bravo	Fabaceae
Schinus terebinthifolius Raddi.	Aroeira-pimenteira	Anacardiaceae
Tabermaemontana hystrix Steud.	Leiteiro-Jasmin	Apocynaceae
	Secondary species	
Cecropia hololeuca Miq.	Embaúba-prateada	Urticaceae
Citharexylum myrianthum Cham.	Pau-Viola	Verbenaceae
Joannesia princeps Vell. LC.	Boleira	Euphorbiaceae
Peltophorum dubium (Spreng) Taub.	Canafístula	Fabaceae
Psidium guineense Sw.	Araçá-do-campo	Myrtaceae

Plant seeds were germinated in sand and seedlings with the first pair of true leaves were transplanted to tubes (180 cm^3) containing the substrates Ouro Verde or Antuérpia. Composition, chemical and physical analyses (Table 02) of both substrates were made

according to the normative instruction of the Brazilian Ministry of Agriculture, Livestock and Supply.

Table 2. Chemical and physical characterization of the Antuérpia and Ouro Verde substrates used for the production of seedlings of 13 native tree species from the Atlantic Forest of Brazil.

Substrates	**pH*** (H_2O)**	**N**	**P**	**K**	**Mg**	**OC*** **	**C/N*** **	**WHC*** ** **(-10 Kpa)**	**TD*** **	**EC*** **
				%				(kg/kg^{-1})	($g.cm^{-3}$)	(mS/cm^{-1})
Ouro Verde Substrate*	8.50	0.42	0.28	0.32	0.23	3.43	8.16	0.46	0.69	2.88
Antuérpia Substrate**	6.04	0.73	0.47	1.28	1.37	6.55	8.97	0.99	0.45	0.66

**Substrate based on 75% organic compost, composed of eucalyptus husk and carbonized rice husk + 25% vermiculite*

***Substrate based on 80% organic compost based on sugarcane bagasse, chicken litter and coal mill + 20% Red -yellow Dystrophic Latosol*

****pH: hydrogen ion potential in a substrate solution: water (1:2.5 v/v); N: Kjeldahl method (substrate KCl extraction followed by acid digestion); P and K: Mehlich-1 extractor; Mg2+: extracted with 0.1 N KCl; OC: Organic carbon the Walkley-Black method; C/N: carbon:nitrogen ratio; WHC: water retention capacity under 10 kPa tension; TD: total density (dry mass per volume unit); E.C: electrical conductivity (obtained by the saturated paste extract).*

At transplanting, seedlings were inoculated or not with 15 mL (150 spores/mL) of a multi-specific mycorrhizal inoculum produced using the on-farm method (Moreira et al. 2019; Prado et al. 2021). The origin of AMF propagules to produce the on-farm inoculum was soil from an Atlantic Forest area located at district of Paracatu de Baixo, in Mariana, Minas Gerais, Brazil. To produce the on-farm inoculant, forest soil, sugarcane bagasse, and vermiculite were mixed in the proportion 1:1:1 and 10% of goat manure were added to the final mix volume. The mixture was placed in plastic buckets with a capacity of 20 L, with perforations at the bottom to drain excess of water, and seeded with *Sorghum bicolor* L. (average of 12 plants per bucket). Buckets were maintained outdoors in a farm, irrigating as necessary for 120 days, when irrigation was suppressed to stimulate sporulation. Evaluation of the on-farm inoculum revealed spores of following AMF species: *Acaulospora scrobiculata* Trappe, *Paraglomus albidum* cf. C. Walker & L.H. Rhodes, *Paraglomus brasilianum* (Spain & J. Miranda) J.B. Morton & D. Redecker, *Paraglomus* sp., *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler. After transplanting, seedlings were cultivated in a commercial nursery in full sun and received daily irrigation by microsprinkler for 7 min every hour from 8 a.m. to 4 p.m. Irrigation varied according to the climatic conditions of the day. The experiment was conducted in a 2 x 2 factorial arrangement for each of 13 tree native species. Treatments consisted of substrate (Antuérpia and Ouro Verde) and mycorrhizal inoculation (with or without on-farm

inoculum), and they were arranged in a completely randomized design, with five replicates per treatment, totaling 260 experimental units.

Morphological characteristics of the evaluated plant species

After 150 days of the onset of the experiment, seedlings height and stem diameter were measured using a ruler and digital caliper, respectively. Seedlings shoots were cut at substrate level, acconditioned in paper bags and dried in a forced circulation oven at 65 ºC until constant mass to obtain shoot dry biomass. The root system was washed in tap water, cut into pieces of about 2 cm, and stored in a 50% alcohol solution for further evaluation of mycorrhizal colonization.

Mycorrhizal Colonization

The root system was cleared with 10% KOH (w/m). As the roots of the 13 species were highly pigmented, an extra step was necessary using a 10% KOH + 10% H2O2 (v/v) solution in a 1:1 (v:v) and subsequent staining using trypan blue to 0.05% in lactoglycerol (Phillips and Hayaman, 1970 modified). After staining, the roots were stored in a lactoglycerol solution (Brundrett et al. 1996). The stained roots were observed under a dissecting microscope and the percentage of mycorrhizal colonization was estimated by the grid-line intersect method (Giovannetti and Mosse 1980) by inspecting 100 intersections per root system.

Statistical Analysis

Data were analyzed for normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test) using R software. Data on mycorrhizal colonization was arcosine ($\sqrt{x}/100$) transformed. The height, stem diameter and shoot dry mass and mycorrhizal colonization data were submitted to analysis of variance (ANOVA), and when significant, the means were compared by Tukey test at 0.05% and the analyzes were performed using the ExpDes.pt package (Ferreira et al. 2018). All mentioned analyzes were performed with the Rstudio version 4.0.3 program (R core Team, 2020).

Results

In general, the pioneer species had their height and shoot dry biomass influenced by the substrate and by the mycorrhizal inoculation and later by the interaction of both factors, while

the stem diameter was affected mainly by the type of substrate (Table 3). Mycorrhizal colonization of pioneer species, were affected by the substrate, except for *Albizia niopoides* and *P. nitens*, which inoculation and the interaction between factors influenced this variable (Table 3). Seedlings of *A. niopoides*, *Schinus terebinthifolius*, *Pterogyne nitens*, *Anadenanthera peregrina*, *Tabermaemontana hystrix* and *Colubrina glandulosa*, when grown in Antuérpia substrate, presented higher height and dry biomass and stem diameter compared to those grown in the Ouro Verde substrate. The addition of inoculum to seedlings for *A. peregrina* had a negative effect, producing plants with lower height, stem diameter and shoot biomass, while for *A. niopoides*, presented higher height when inoculated (Table 3). Plants of *S. terebinthifolius*, *G. ulmifolia* and *P. reticulata* produced higher shoot biomass when the seedlings were not inoculated. When there is interaction between the factors, the substrate Antuérpia, when inoculated, promotes greater height in seedlings of *G. ulmifolia* and greater stem diameter in *S. terebinthifolius* compared to Ouro Verde (Table 3). On the other hand, addition of AMF inoculum to the Ouro Verde substrate did not increase the height of *G. ulmifolia* and stem diameter in *P. reticulata* seedlings in relation to plants without inoculation.

All seedlings were colonized by AMF and mycorrhizal colonization rates ranged from 8 to 95.8% in pioneer plants (Table 3). The substrates influenced the mycorrhizal symbiosis in seedlings of *A. peregrina*, *C. glandulosa* and *S. terebinthifolius* with a higher percentage of colonization observed in the substrate Antuérpia in relation to Ouro Verde. When we observed only the effect of mycorrhizal inoculation, we found that the addition of the inoculum in *A. niopoides*, *P. reticulata* and *T. hystrix* favors colonization in relation to the non-inoculated treatment. However, seedlings of *A. niopoides* and *P. nitens* growing in both substrates, when inoculated, increased the percentage of mycorrhizal colonization in relation to their control (Table 3).

The interaction between the factors influenced the height in most secondary species, followed by the factors tested separately, except for *P. guineense* which was influenced solely by inoculation and *C. hololeuca* by the type of substrate for this parameter (Table 3). Regarding to stem diameter and shoot biomass, the most secondary plants were influenced by both factors, substrate and mycorrhizal inoculation, in isolation, except for *J. princeps* and *C. hololeuca* that had their stem diameter affected, mainly by the substrate, while *C. myrianthum* had its biomass modulated as a function of inoculation (Table 3). However, mycorrhizal colonization in secondary species was affected by the interaction of factors and in isolation, except for *P. guineense*, in which the substrate affected this parameter (Table 3).

Highest height was observed in *C. hololeuca*, *C. myrianthum*, *P. dubium* and *P. guineense* by the addition of the inoculum in the Antuérpia substrate, while the inoculation negatively affected this parameter in the Ouro Verde substrate for the last three plants, except for *C. hololeuca* which inoculation did not (Table 3). When observed alone, the Antuérpia substrate in *P. dubium*, *P. guineense* and *C. hololeuca* seedlings increased the stem diameter when compared to the Ouro Verde substrate. However, this parameter was also negatively affected by the addition of mycorrhizal inoculant in *P. dubium* and *P. guineense* seedlings (Table 3). In *J. princeps* seedlings there was an interaction between the factors, but in the Antuérpia substrate, in the presence of the inoculant, is the most favorable in promoting the stem diameter. The shoot dry biomass was higher in *P. dubium* and *P. guineense* seedlings in the Antuérpia substrate when compared to the Ouro Verde substrate, in addition, mycorrhizal inoculation negatively affected the shoot dry biomass of these species (Table 3). On the other hand, in *C. hololeuca* seedlings, the substrate Antuérpia, when inoculated, promotes greater production of dry biomass in relation to the substrate Ouro Verde. Only *C. myrianthum* had its shoot dry biomass affected by the mycorrhizal inoculation, were inoculated treatment presented lower value in relation to the non-inoculated control.

For secondary species, all seedlings were also colonized, and mycorrhizal colonization ranged from 29.6 to 90.4 % (Table 3). Most of the studied plants showed interaction between the factors and for *P. dubium* and *J. princeps* when they were not inoculated, the Antuérpia substrate promotes greater mycorrhizal colonization in relation to Ouro Verde, while when both substrates were inoculated, they increase the mycorrhizal colonization in *C. myrianthum* seedlings (Table 3). Only *P. guineense* had its mycorrhizal colonization affected by the substrate, with Antuérpia being the most indicated compared to Ouro Verde.

Table 3. Height, stem diameter, shoot dry mass and mycorrhizal colonization of 13 seedlings of native Atlantic Forest species produced in Antuérpia and Ouro Verde substrates, inoculated (With) or not (Without) with FMA after 150 days of cultivation in commercial nursery conditions.

Substrates	**Height (cm)**		Average	**Stem diameter (mm)**		Average	**Shoot dry biomass (g)**		Average	**Mycorrhizal colonization (%)**		Average
	Without	**With**		**Without**	**With**		**Without**	**With**		**Without**	**With**	
						Pioneer species						
						Albizia niopoides						
Ouro Verde	8.58	16.46	16.81b	3.11	2.79	2.95b	1.02	0.81	0.91b	18.0aB	28.0bA	23.0
Antuérpia	34.00	42.98	38.49a	4.37	5.19	4.78a	4.35	4.78	4.57a	13.0aB	38.8aA	25.9
Average	21.29b	29.72a		3.74	3.99		2.68	2.79		15.5	33.4	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	10.04	0.01		0.76	ns		0.04	ns		39.39	<0.001	
Substrate	95.33	<0.001		11.75	<0.001		78.69	<0.001		1.03	ns	
I X S	0.04	ns		1.15	ns		1.17	ns		7.67	0.01	
						Anadenathera peregrina						
Ouro Verde	18.36	18.04	18.20b	2.81	2.50	2.66b	1.15	0.46	0.81	66.0	67.2	66.6b
Antuérpia	26.82	20.24	23.53a	3.21	3.28	3.24a	1.04	0.69	0.87	80.2	96.2	88.2a
Average	22.59a	19.14b		3.01	2.90		1.10a	0.58b		73.1	81.7	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	5.29	0.05		0.47	ns		17.69	<0.001		0.72	ns	
Substrate	12.64	<0.001		10.72	0.01		0.21	ns		4.55	0.05	
I X S	4.36	ns		1.06	ns		1.92	ns		0.53	ns	
						Colubrina glandulosa						
Ouro Verde	14.84	12.56	13.70b	4.68	5.35	5.02b	1.53	1.61	1.57b	50.8	69.4	60.1b
Antuérpia	17.24	15.84	16.54a	5.67	5.57	5.62a	2.34	2.29	2.32a	94.6	95.8	95.2a
Average	16.04	14.20		5.18	5.46		1.94	1.95		72.7	82.6	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	10.84	ns		1.46	ns		0.01	ns		3.98	ns	
Substrate	25.73	<0.001		6.44	0.05		16.32	<0.001		4.67	0.05	
I X S	0.62	ns		2.56	ns		0.13	ns		0.05	ns	
						Guazuma ulmifolia						
Ouro Verde	5.81aA	4.66bB	5.24	5.60	4.57	5.08b	3.14	1.72	2.43	52.6bB	74.2aA	63.4
Antuérpia	5.98aA	5.64aA	5.81	6.02	6.33	6.17a	2.99	2.67	2.83	81.6aA	80.0aA	80.8

Average	5.90	5.15		5.81	5.45		3.06a	2.19b		67.1	77.1	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	15.40	0.01		0.95	ns		6.66	0.05		3.85	ns	
Substrate	9.46	0.01		8.58	0.01		1.40	ns		9.83	0.01	
I X S	4.72	0.05		3.26	ns		2.68	ns		4.86	0.05	
					Plathymenia reticulata							
Ouro Verde	50.84aA	32.20bB	41.52	7.18aA	5.76aB	6.47	8.97	6.12	7.55	44.2	51.6	47.9
Antuérpia	44.88aA	40.20aA	42.54	6.15aA	6.20aA	6.18	7.42	6.55	6.99	37.0	58.2	47.6
Average	47.86	36.20		6.67	5.98		8.20a	6.34b		40.6b	54.9a	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	5.30	<0.001		0.89	0.05		17.68	<0.001		4.55	0.05	
Substrate	12.64	<0.001		4.88	ns		0.21	ns		0.72	ns	
I X S	4.36	ns		5.56	0.05		1.92	ns		0.53	ns	
					Pterogyne nitens							
Ouro Verde	8.58	8.54	8.56b	3.11	3.08	3.10	1.02	0.62	0.82b	23.8bB	81.0aA	52.4
Antuérpia	10.10	9.96	10.03a	3.48	3.14	3.31	1.17	0.96	1.07a	89.0aA	95.6aA	92.3
Average	9.34	9.25		3.30	3.11		1.10a	0.79b		56.4	88.3	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	0.02	ns		1.63	ns		11.23	<0.001		13.68	<0.001	
Substrate	5.91	0.05		2.34	ns		6.94	0.01		21.40	<0.001	
I X S	0.01	ns		1.34	ns		1.14	ns		8.60	0.01	
					Schinus terebinthifolius							
Ouro Verde	34.46	26.46	30.46b	7.06aA	6.53bA	6.79	5.16	3.38	4.27b	85.8	94.6	90.2b
Antuérpia	44.26	33.76	39.01a	6.89aA	7.69aA	7.29	5.79	5.24	5.51a	90.7	95.8	93.2a
Average	39.36a	30.11b		6.98	7.11		5.48a	4.86b		88.2	95.2	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	19.29	<0.001		0.21	ns		6.05	0.05		1.95	ns	
Substrate	16.48	<0.001		2.67	ns		6.85	0.05		15.17	0.01	
I X S	0.35	ns		4.86	0.05		1.66	ns		0.30	ns	
					Tabermaemontana hystrix							
Ouro Verde	8.4	8.96	8.68b	3.33	3.25	3.29	0.44	0.31	0.38b	8.0	54.2	31.1
Antuérpia	14.22	12.88	13.55a	3.94	3.55	3.75	0.95	0.60	0.78a	37.0	40.2	38.6
Average	11.31	10.92		3.64	3.40		0.69	0.46		22.5b	32.2a	

	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	0.06	ns		0.86	ns		3.81	ns		2.59	ns	
Substrate	10.10	0.01		3.18	ns		10.73	0.01		12.59	0.01	
I X S	0.38	ns		0.38	ns		0.71	ns		1.83	ns	
Secondary species												
Cecropia hololeuca												
Ouro Verde	16.64aA	13.12bA	14.88	5.12	5.75	5.43b	1.73aA	1.68bA	1.70	57.2	71.0	64.1
Antuérpia	15.58aB	23.52aA	19.55	6.01	6.75	6.38a	1.68aB	2.66aA	2.17	71.0	78.2	74.6
Average	16.11	18.32		5.56	6.25		1.70	2.17		64.1	74.6	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	2.26	ns		2.62	ns		6.74	0.01		1.44	ns	
Substrate	10.11	0.01		5.04	0.05		6.92	0.01		1.44	ns	
I X S	15.21	<0.001		0.02	ns		8.35	0.01		0.76	ns	
Cytharexylum myrianthum												
Ouro Verde	33.06aA	22.36bB	27.71	8.74	7.63	8.18	6.74	4.13	5.44	29.8bB	68.0bA	48.9
Antuérpia	33.16aA	31.80aA	32.48	8.71	9.06	8.88	7.50	5.97	6.74	74.2aB	90.4aA	82.3
Average	33.11	26.77		8.72	8.35		7.12a	5.05b		52.0	79.2	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	13.85	<0.001		0.79	ns		9.06	0.01		33.01	<0.001	
Substrate	8.67	0.01		2.71	ns		3.58	ns		49.78	<0.001	
I X S	8.31	0.01		2.93	ns		0.61	ns		5.39	0.05	
Joannesia princeps												
Ouro Verde	39.74	34.70	37.22	12.25aA	10.99bA	11.62	10.11	6.74	8.42	29.6bB	76.0aA	52.8
Antuérpia	40.68	38.12	39.4	12.43aB	14.95aA	13.69	9.96	11.89	10.93	77.4aA	86.4aA	81.9
Average	40.21	36.41		12.34	12.97		10.04	9.32		53.5	81.2	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	1.08	ns		0.57	ns		0.30	ns		16.68	<0.001	
Substrate	0.35	ns		6.32	0.05		3.59	ns		17.93	<0.001	
I X S	0.11	ns		5.26	0.05		4.03	ns		5.14	0.05	
Peltophorum dubium												
Ouro Verde	20.24bA	14.08bB	17.16	5.49	6.64	6.07b	3.68	1.44	2.56b	42.8bB	86.0aA	64.4
Antuérpia	30.24aA	19.84aB	25.04	6.54	7.62	7.08a	5.44	2.56	4.00a	77.2aA	86.0aA	81.6
Average	25.24	16.96	21.10	6.01b	7.13a		4.56a	2.00b		60.0	86.0	

	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	85.67	0.01		17.67	<0.001		50.53	<0.001		10.89	0.01	
Substrate	78.09	<0.001		14.54	<0.001		15.86	<0.001		4.76	0.01	
I X S	7.83	0.01		0.88	ns		0.79	0.387		4.77	0.05	
					Psidium guineense							
Ouro Verde	23.46aA	16.94bB	20.2	5.22	3.54	4.38b	4.92	2.08	3.50b	32.8	57.2	45.0b
Antuérpia	27.80aA	21.90aA	24.85	5.72	4.48	5.10a	5.84	3.92	4.88a	76.0	79.8	77.9a
Average	25.63	19.42		5.47a	4.01b		5.38a	3.00b		54.4	68.5	
	F Value	*P*		F Value	*P*		F Value	*P*		F Value	*P*	
Inoculation	17.34	<0.001		19.77	<0.001		18.55	<0.001		3.73	ns	
Substrate	0.04	ns		4.77	0.05		6.20	0.05		20.32	<0.001	
I X S	9.72	0.01		0.46	ns		0.71	ns		1.99	ns	

Means followed by different lowercase letters in the column and uppercase letters in the row differ statistically from each other by the T-Student test at 5%.

Discussion

These are the first results on the effect of substrate and AMF on the growth of native tree species of Atlantic Forest, destined for revegetation of areas affected by the rupture of the Fundão dam in the Rio Doce Basin. Other studies carried out in Brazil have shown that pioneer species are much more responsive to mycorrhizal inoculation than secondary species (Carneiro et al. 1996; Zangaro et al. 2003). However, this AMF inoculation response in the different successional groups is still unclear, as there are controversies in the literature, which have been reported that secondary species are more benefited by inoculation compared to early growth species (Koziol and Bever, 2016). On the other hand, we generally observed that both successional groups did not respond positively to inoculation when its effect was observed alone, with the exception of *A. niopoides* which presented greater height when inoculated in relation to the treatment without inoculation. There is little information about the growth of seedlings of *A. niopoides*, and nothing is known about the effect of inoculation with symbiotic microorganisms on the growth of this forest legume species. However, inoculation of seedlings of other genera of *Albizia* has been tested (Carneiro et al. 1996; Siqueira et al. 1998; Santos et al. 2016). A study carried out with *Albizia saman* (Jacq.) Merr., a tropical tree species of the Fabaceae family, showed that seedlings inoculated and cultivated under nursery conditions are dependent on inoculation to promote their growth (Wulandari et al., 2014; Wulandari et al., 2016), corroborating with what we have observed for *A. niopoides*, which leads us to believe that species of this genus are really benefited and respond positively to inoculation with AMF.

Mycorrhizal inoculation in seedlings of *S. terenbithifolius* and *A. peregrina* reduced the growth of these tree species, diverging from other results reported in the literature, that these plants are highly responsive to inoculation (Zangaro et al. 2000; Pasqualini et al. 2007; Goetten et al. 2016), due to its ability to associate with a large number of symbiotic and beneficial microorganisms (Dawkins and Esiobu, 2017). Similar to the results found by Carneiro et al. (1996), we confirmed that the secondary species *P. dubium* did not respond to mycorrhizal inoculation in a nursery. While, *C. myrianthum* had at least one of its growth parameters reduced as a function of inoculation, which is the opposite of what was observed by Pasqualini et al. (2007), in which all growth parameters of this plant were responsive to the addition of the inoculum. In seedlings of *C. hololeuca*, *J. princeps* and *P. guineense* (secondary) and *T. hystrix* (pioneer) inoculation had a neutral effect on all plant development parameters. According to Carneiro et al. (2004) *C. hololeuca* seedlings are considered facultative mycotrophs, so we can suggest that the availability of nutrients present in the tested substrates may have influenced the

inoculum efficiency. Furthermore, we did not find any reports in the literature on the effect of AMF inoculation on the development of *P. guineense* seedlings. However, in species belonging to the Myrtaceae family beneficial effects of inoculation with native AMF on height and dry biomass of the aerial part of *P. guajava* has been observed (Scabora et al. 2010). However, in the present study it is evident that the mycorrhizal inoculation brought little increment in the initial development independent of the successional group, may be species of inoculated fungi can also result in divergent results.

Linked to these factors, the substrates tested were not sterilized, that is, they already had a resident AMF community and adapted to the physical and chemical conditions of the tested substrates, and it is possible that the action of these AMF was the same or higher than that observed in the treatments. inoculated. Silva and Silva (2017) also did not observe greater growth in the tree species of *Mimosa tenuiflora* (Wild.) Poir. when inoculated with AMF in native soil (not sterilized), not finding benefits of inoculation in growth parameters. Furthermore, it is known that the use of autochthonous inoculants is more beneficial for native tree species compared to allochthonous AMF inoculants, with the origin of the inoculant being an important predictor of plant community (Hart et al. 2018), so it is possible that AMF do not always stimulate plant growth when the cost of drained carbon exceeds the benefits of nutrient uptake via symbiosis (Bâ et al. 2001; Klironomos, 2003).

Among the many factors that affect seedling production, the physical and chemical composition of substrates is one of them (Pascual et al. 2018; Oliveira Júnior et al. 2019; Abaurre et al. 2020; Afonso et al. 2020). Therefore, interest has grown in the use of alternative technologies that improve the physical and chemical conditions (Afonso et al. 2020), and consequently the biological conditions of the substrates during the seedling production phase. A good substrate should be one capable of promoting a greater speed of seedling growth and development, in order to avoid exposing the seedlings to adverse conditions in the seed phase (Afonso et al. 2020). Our study clearly demonstrates that the species of the pioneer successional group were the most influenced by the type of substrate, while for the secondary species only *P. guineense* and *P. dubium* had at least one of their growth parameters affected. As the pioneer species are fast-growing plants with high nutritional demand, the physical and chemical composition has a greater effect on the species with this developmental habit, while with the advancement of the successional group the responses to fertility are less pronounced (Santos et al. 2008; Berti et al. 2017). However, under conditions of greater fertility, responses to growth parameters as a function of mycorrhizal inoculation are reduced (Vandresen et al. 2007).

Alternatively, the greater influence of the type of substrate on the pioneers compared to the secondary ones can also be attributed to the morphological characteristics of the roots. Pioneer species have a more developed root system with thinner and branched roots in relation to late groups, which makes these plants able to grow, exploit and more efficiently absorb the nutrients available in the substrates (Gonçalves et al. 1992).

Thus, the Antuérpia substrate, due to its physical composition such as greater water retention capacity, lower total density and low electrical conductivity and better nutritional conditions than Ouro Verde, was the substrate capable of providing greater development in pioneer seedlings and in some secondary species regardless of inoculation. In seedlings of *T. catharinensis*, a pioneer species, Afonso et al. (2020) also found that the physical and chemical composition of substrates consisting of 50% commercial compost +50 or 75% vermiculite promoted greater water retention capacity, lower electrical conductivity and density, providing the seedlings with greater vigor and growth, its use for seedling production is recommended. In addition, the low development of seedlings in the Ouro Verde substrate can be attributed to greater compaction in relation to the Antuérpia substrate, reducing gas exchange, associated with low nutritional conditions due to the pH being highly alkalized, making nutrients unavailable could explain this result, corroborating with observed by (Abaurre et al. 2021).

The choice of cultivation substrate used for the production of forest seedlings influences the responsiveness of mycorrhizal inoculation (Abaurre et al. 2020). The substrate that promotes greater plant growth is not always the most promising for the formation of mycorrhizal symbiosis, as observed in *S. saman* seedlings (Abaurre et al. 2020 and 2021), which differs from the present study for the Antuérpia substrate. All species of the pioneer and secondary successional groups showed mycorrhizal colonization rates when inoculated above 20%, in agreement with the study by Carneiro et al. (1996). The seedlings of *S. terebinthifolius*, *A. peregrina*, *C. glandulosa* (pioneer) and *P. guineense* (secondary) when produced in the Antuérpia substrate favored the formation of symbiosis with AMF, in addition to promoting greater seedling development. This higher percentage of mycorrhizal colonization in this substrate can be explained by its better physical conditions, such as, for example, the higher water buffering capacity. Similarly, Abaurre et al. (2020) also noted that substrates that have a greater water-buffering capacity stimulate the plant to form a symbiosis with AMF to go in search of water. However, it was evident that most seedlings, when inoculated and produced in Ouro Verde substrate, had mycorrhizal colonization stimulated. However, we observed a low response of native tree seedlings to inoculation, which contrasts with mycorrhizal colonization,

which was considered medium to high for most tree species studied according to the classification by Carneiro et al. (1996). Vandresen et al. (2007) found a low response of *Bastardiopsis densiflora* (Hook. & Arn.) Hassl., *Croton urucurana* Baill., *Senna macranthera* (Collad.) H.S. Irwin & Barneby and *Bauhinia forficata* Link against inoculation, but all species showed a percentage of mycorrhizal colonization of 54 to 77%. According to these authors, this low response of plants to inoculation is due to the rare presence of arbuscules (site of exchange of nutrients) in the roots of the plants and more presence of fungal hypha, which may also have happened in our study although we did not quantify the structures produced by the FMA. When observing the isolated effect of the mycorrhizal inoculation, only the species *P. reticulata* and *T. hytrix*, both pioneers, had their percentage of mycorrhizal colonization increased by the addition of the inoculum. *P. reticulata* seedlings are known to be colonized by AMF, being highly responsive to inoculation (Prates Júnior et al. 2021), however, we have seen that their responsiveness will depend on the type of substrate.

Conclusion

The Antuérpia substrate provides a higher development, mainly of tropical native pioneer species, in addition to being the most conducive to symbiosis allowing greater mycorrhizal colonization. Pioneer habitat species are more susceptible to the type of substrate used for their production in the nursery. The type of substrate limits the benefits of AMF association in the production of native tree seedlings, and further studies are recommended in search if this substrate reflect in the growth an establishment of plant in field.

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4. Chapter 2: Written according to Mycorrhiza

The community of native AMF in the rhizosphere of tropical tree species is impacted by plant species and substrate characteristic

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Abstract

Mycorrhizal inoculation with arbuscular mycorrhizal fungi (AMF) can improve the success and establishment of native forest species for ecological restoration. However, mycorrhizal inoculation and cultivation substrates can impact the structure of the native AMF community by affecting plant performance during seedling production. The aim of this study was to investigate if the introduction of AMF isolates and the cultivation substrate promote changes in the structure of the native mycorrhizal community present in the rhizosphere of six tropical tree species and if there is preferential host selection by some AMF species. The number of glomerospores varied among the tree species studied, and by the cultivated substrate, where in general, inoculation and Antuérpia substrate increased AMF sporulation. Twenty-nine AMF species were recorded in the rhizosphere of tree species, with a predominance of species of the genus *Acaulospora*, *Glomus* and *Paraglomus* regardless of the substrate. The richness, Shannon diversity and evenness were favored by inoculation in seedlings of *Schinus terebinthifolius* and *Colubrina glandulosa*. However, the Antuérpia substrate increases the dominance in seedlings of *Tabermaemontana hystrix*, as demonstrated by the main coordinate analysis (PCoA) and PERMANOVA. *Acaulospora delicata, Claroideoglomus etunicatum* and *Glomus* sp.1 served as indicators for *S. terebinthifolius* and *Acaulospora* sp. 2 for *C. glanduolosa* and *Plathymenia reticulata*. While *Acaulospora* sp.1 and *Funneliformis mosseae* were indicators of the inoculation treatments. *C. etunicatum* was found treatments and can be considered a generalist fungus. It is concluded that the introduction of AMF inoculum alters the structure of native

AMF community, with lesser effect of the cultivation substrate and that native tree species are select specific fungal groups of AMF.
Keywords: Mycorrhizal diversity, arbuscular mycorrhizal fungi, native plant, inoculation, substrate

Introduction

Forests are responsible for hosting most of the world's biodiversity and therefore the conservation of this habitat is totally dependent on how we use it (FAO, 2020). The reduction of this ecosystem is directly associated with anthropic actions, such as the conversion of forest areas to pastures, climate change, land use, environmental disasters, economic expansion (Pawson et al. 2013; Lawrence and Vandecar, 2015; Prado et al. 2019) and population that has led to forest fragmentation of natural assets affecting the functioning of ecosystem services (Hasan et al. 2020). In this context, the ecological restoration of the soil is necessary, whose objective is to recover an environment that was degraded, damaged, and destroyed (Seri, 2004). The recovery of a heavily degraded environment requires the incorporation of soils with active biological characteristics. Among the strategies, the introduction of seedlings associated with the presence of soil microorganisms beneficial to the growth media can be the initial step in the restoration process, mainly aiming at reducing costs with the use of mineral fertilizers and a more ecologically correct agriculture (Pascual et al. 2018). Thus, it is of great importance to recover areas to reverse this scenario through the reintroduction of native forest seedlings with the presence of beneficial microorganisms (Asmelash et al. 2021).

A well-established association between symbiotic plants and microorganisms is arbuscular mycorrhiza, that is formed by the of arbuscular mycorrhizal fungi (AMF) (Smith and Read, 2008). This symbiosis is considered an essential mutualistic interaction for the initial process of production and establishment of seedlings in nurseries and fields (Berruti et al. 2016), helping in the initial stages of plant succession (Carneiro et al. 1996; Zangaro et al. 2002) playing an important role in nutrient uptake. in addition to improving soil aggregation (Rillig, 2004). However, substrates inoculation with AMF can lead to changes in the composition of microbial communities residing in the rhizosphere of plants, reflecting on their performance (Mummey et al. 2009; Lance et al. 2019), and consequently on the composition of the plant community (van der Heidjen et al. 1998). Furthermore, there are reports that the introduction of exotic inoculants negatively affects the richness and diversity of AMF in relation to the native inoculum in seedlings of *Tamarix articulata* (Bencherif et al. 2021), as well as the type of host

plant and the management adopted that may reflect changes in the structure of the FMA community (Pagano et al. 2011).

In addition, one of the main costs involved in the production of seedlings in nurseries is labor, which can reach around 59% of costs, followed by the substrate used for vegetative growth, which can reach up to 23% of production costs (Pascual et al. 2018), the latter being the main obstacle to failure in the initial stages of plant growth in nurseries, and consequently, in the establishment of planted forests in the field. However, due to the high costs of commercial substrates in the market, nurseries were encouraged to seek alternative technologies as a means of growth for seedlings in nurseries (Abad et al. 2001; Ortuño et al. 2018), being the choice of material a critical factor to ensure optimal plant growth (Pascual et al. 2018). Thus, the cultivation substrates must meet the ideal requirements for the growth of seedlings in their early stages, as such conditions can positively or negatively affect the morphological and nutritional parameters of the plants (Camara et al. 2017). In addition to influencing plant performance recently, Abaurre et al. (2020) found that the physical characteristics of the substrates influenced the success of mycorrhizal symbiosis in *Samanea saman* substrates inoculated with a mix of AMF species. Moreover, spore density is affected by physical and chemical factors present in the "soil" cultivation environment (Silva-Flores et al. 2019). Although, the composition of substrates can influence different forest species as observed by Oliveira Junior et al. (2019). However, there are few studies that assess the impact of inoculation, the cultivation substrate, as well as whether the host plant can lead to changes in the structure of the AMF community are still scarce in native tree species produced in nurseries.

Thus, the present study tested the hypothesis that mycorrhizal inoculation with introduced AMF strains and the culture substrate alters the resident AMF assemblage, resulting in variations in the number of glomerospores. We also tested the hypothesis that these changes caused by inoculation may lead native tree species to form preferential associations with some specific AMF species through indicator species analysis. Thus, the aim of this study was to investigate whether the introduction of exotic AMF isolates and the cultivation substrate promote changes in the native AMF community in the rhizosphere of six native tropical tree species and whether plant hosts preferentially elect some AMF species.

Material and methods

Experimental conditions

The experiment was carried out from September 2019 to February 2020 (150 days) in the Ouro Verde commercial nursery (https://www.facebook.com/ouroverde.viveiro/), located in the city of Belo Oriente, Minas Gerais, Southeast of Brazil (19°11'39.7"S 42°24'50.9"W). The region's climate is of the Aw type, according to Koppen's classification, with an annual average temperature of around 21.4 ºC.

For the present study, six native tree species from the Atlantic Forest Biome were chosen based on a list made available by the Renova Foundation, whose objective was to produce seedlings to be used in the recovery of the Rio Doce Basin, an environment that was affected in November 2015 by the collapse of the Fundão dam in Mariana, Minas Gerais. The selected native plant species were described and classified according to the successional group as early pioneer (Table 1).

Table 1. Native tree species of the Atlantic Forest Biome selected to produce seedlings using different substrates and mycorrhizal inoculation.

Species	**Brazilian common Name**	**Family**
Colubrina glandulosa Perkins	Saguaraji	Rhamnaceae
Joannesia princeps Vell. LC.	Boleira	Euphorbiaceae
Plathymenia reticulata Benth.	Vinhático	Fabaceae
Pterogyne nitens Tul.	Amendoim-bravo	Fabaceae
Schinus terebinthifolius Raddi.	Aroeira-pimenteira	Anacardiaceae
Tabermaemontana hystrix Steud.	Leiteiro-Jasmin	Apocynaceae

Seedlings production

Tubes with 180 cm3 capacity were used as containers for the production of seedlings. Two substrates were used (Table 2): 1) obtained and produced in the Ouro Verde nursery (Belo Oriente); 2) developed in the Antuérpia nursery (Viçosa). For the production of seedlings, the seeds of the native species were put to germinate in sand and the seedlings that presented the first pair of true leaves were selected. then transplanted to the tubes containing the substrates, inoculated or not with 15 mL (150 spores/mL) of a mix of AMF species obtained from Cogumê Biotecnologic, a family enterprise. The mix used as inoculant was produced by the on-farm method, whose initial AMF propagules were obtained from forest soil samples from the region close to the disaster site of the Fundão dam failure in the district of Paracatu de Baixo. in Mariana, Minas Gerais, Brazil. To produce the inoculant. a mixture of sugarcane bagasse, vermiculite in the proportion 1:1:1 (v:v:v) plus 10% of goat manure was made. The inoculant

was produced in buckets with a capacity of 20 L with perforations at the bottom to drain excess water and the host used was *Sorghum bicolor* L., with an average of 12 plants per bucket being kept. being produced in the rural area of the municipality of Cajuri, Minas Gerais (-20°18'8.73"S 43°14'11.13"W). The inoculant consisted of the following species: *Acaulospora scrobiculata* Trappe, *Paraglomus cf albidum* C. Walker & L.H. Rhodes, *Paraglomus brasilianum* (Spain & J. Miranda) J.B. Morton & D. Redecker, *Paraglomus* sp., *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler.

Chemical and physical characterization of substrates

The chemical analyzes of the substrates were carried out in the soil fertility laboratory, located in the municipality of Viçosa, Minas Gerais. and the physical analyzes were carried out in the soil physics laboratory of the Federal University of Viçosa (UFV) in accordance with normative instruction nº17 of May 24. 2007 (Brazil 2007) of the Ministry of Agriculture, Livestock and Supply (MAPA/SDA).

The chemical and physical analyzes performed were water holding capacity (WHC) under a tension of 10 kPa, total density (dry mass per volume unit), electrical conductivity (obtained by the saturated paste extract), pH (hydrogenionic potential). nitrogen. phosphorus. potassium. organic carbon and carbon:nitrogen ratio of each substrate. The descriptions of methods and detailed information concerning the soil chemical and physical analyzes are presented in Menezes et al. (2022). After transplanting, the seedlings were cultivated for 150 days in a nursery in full sun and received daily irrigation by microsprinkler for 7 min every 1 hour, varying according to the climatic conditions of the day, starting at 8 am and ending at 4 pm.

Glomerospore extraction

50 g of soil from each sample was used to extract the glomerospores using the wet sieving technique, followed by centrifugation in water and 40% sucrose (Gerdemann and Nicolson, 1963; modified Jenkins, 1964), placed in glass plates and counted under stereomicroscope (40x).

Identification of AMF species

For species identification, AMF spores were extracted from the soil and separated by morphotypes and mounted on slides and coverslips containing the reagents PVLG (polyvinyl-

lacto-glycerol) and PVLG + Melzer (1:1 v/v). The morphological characteristics of the glomerospores were observed under a microscope and the identification of the species was carried out based on identification manuals (Schenck and Peréz, 1990; Blaszkoski, 2012) and descriptions presented on webpages of the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) (www.invam.wvu.edu).

Experimental design

In conducting the experiment. a factorial arrangement (2 x 2) x 5 was adopted, for each of the six plant species, two types of substrates: Antuérpia and Ouro Verde, inoculated or not with AMF, arranged in a completely randomized design, with five replications, totaling 120 experimental units.

Statistical analysis

Glomerospore number, AMF species richness (S), Shannon-Wiener diversity index (H') and Pielou evenness (J) were determined in all treatments and per plant. For the analysis of the number of glomerospores it was necessary to transform the data into log (x) before being submitted to analysis of variance (ANOVA). Univariate analyzes of variance (ANOVA) were calculated using the R Studio packages "*multcomp*", "*lsmeans*" and "*ExpDes.pt*" for number of glomerospores and when significant the means were compared by Tukey test at 0.05% (Lenth, 2016; Ferreira et al. 2018; Oksanen et al. 2019). For the ecological indices of AMF species, the nonparametric Kruskal-Wallis test was used with *p values* adjusted by Bonferroni, using the "*agricolae*" package (Mendiburu, 2020). To determine the relationship of species with inoculation, substrate and plant indicator species analysis was used (Dufrene and Legendre, 1997). The significance of the indication value (IndVal) was evaluated by applying the Monte Carlo test with 1000 permutations. The indicator species were those with a significant *p value* ($p<0.05$) and IndVal $\geq 25\%$. as assessed by the "*indicspecies*" package (Cáceres and Legendre, 2009).

The permutational multivariate analysis of variance (PERMANOVA) was applied, based on the Euclidean distance to test whether the AMF community differs between tested treatments, using the "adonis" function in the "*vegan*" package. Principal coordinate analysis (PCoA) was used to calculate dissimilarities in the composition of AMF using the Euclidean distance. The "envfit" function with 999 permutations was used to fit the AMF communities and treatments using the "vegan" package. All mentioned analyzes were performed with the R

Studio version 4.0.3 program (R core Team, 2020). Venn diagrams were constructed using the tool available online (http://jvenn.toulouse.inra.fr/app/example.html) by Bardou et al. (2014).

Results

Number of glomerospores

Based on the analysis of variance there was an interaction between substrates and inoculation regarding the number of glomerospores for the species of *J. princeps*, *P. reticulata*, *C. glandulosa* and *T. hystrix* (Table 2). For the species of *P. nitens* there was an effect of substrate and inoculation, in which the inoculation promoted greater sporulation than the treatment without inoculation. However, the number of glomerospores was greater in Antuérpia that in Ouro Verde substrate. For the species of *C. glandulosa* and *J. princeps* it was found that when inoculated, the Antuérpia substrate presented greater sporulation than in to the Ouro Verde substrate. Within each substrate we observed that in Antuérpia the inoculation promoted a greater number of glomerospores compared to the control and for the substrate Ouro Verde no differences were found. For *J. princeps* we observed that when unioculated, greater sporulation is observed in Ouro Verde substrate compared to Antuérpia substrate, however, inside Antuérpia inoculation promotes a greater number of glomerospores than its uninoculated control.

Meanwhile, *T. hystrix* found that the Antuérpia substrate promoted a greater number of glomerospores than Ouro Verde when inoculated. When observing inside Antuérpia we verified that its inoculation leads to greater sporulation than when there is no addition of the inoculant (without inoculation). On the other hand, *P. reticulata* showed higher sporulation in Antuérpia substrate compared to Ouro Verde. However, within the Ouro Verde substrate, greater sporulation was observed when inoculated then uninoculated one. For *S. terebinthifolius* we did not observe significant differences between substrates and inoculation treatments (Table 2).

Table 2. Number of glomerospores in seedling rhizosphere of six native tree species produced under two substrates, without or with AMF inoculation, after 150 days of cultivation.

Number of Glomerospores (20 g of soil)						
	Pterogyne nitens			***Schinus terebinthifolius***		
Substrates	**Without**	**With**	Average	**Without**	**With**	Average
Ouro Verde	18.00	44.75	31.37b	400.75	469.75	435.25
Antuérpia	41.50	73.50	57.50a	299.50	473.75	386.62
Average	29.75b	59.12a		350.12	471.75	
Substrates	***Joannesia princeps***			***Tabermaemontana hystrix***		
	Without	**With**	Average	**Without**	**With**	Average
Ouro Verde	69.50aA	94.50aA	82.00	30.25aA	48.75bA	39.50

Antuérpia	20.50bB	138.75aA	79.62	32.75aB	138.25aA	103.00
Average	45.00	116.62		31.50	93.50	
Substratos	***Colubrina glandulosa***			***Plathymenia reticulata***		
	Without	**With**	Average	**Without**	**With**	Average
Ouro Verde	20.25aA	21.50bA	20.87	26.00bB	257.00aA	141.50
Antuérpia	32.25aB	156.25aA	94.25	164.50aA	251.75aA	208.12
Average	26.87	88.87		95.25	254.37	

Means followed by different lowercase letters in the column and uppercase letters in the row differ statistically from each other by the T-Student test at 5%.

Ecological indices and mycorrhizal community in the rhizosphere of native tree species after addition of inoculant and different cultivation substrates

A total of 29 AMF taxa belonging to nine families and 12 genera were identified (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Diversisporaceae, Claroideoglomeraceae, Paraglomeraceae, Gigasporaceae (Table 3). *Acaulospora* was the genus with the highest number of species (9), followed by *Glomus* (5), *Paraglomus* (3), *Funneliformis* (2), *Ambispora* (1), *Archaeospora* (1), *Cetraspora* (1) *Claroideoglomus* (1), *Diversispora* (1), *Rhizophagus* (1), *Scutellospora* (1) and *Septoglomus* (1) (Table 3).

Acaulospora delicata (IV = 0.536. p = 0.0001), Claroideoglomus etunicatum (IV = 0.617. p= 0.0001) and *Glomus* sp.1 *(IV = 0.268. p= 0.04)* were selected as indicator species of the tree species *S. terebinthifolius.* While *Acaulospora* sp.2 *(IV = 0.346. p = 0.02)* was considered to indicate the species *P. reticulata* and *Glomus* sp.1 *(IV = 0.268. p = 0.04)* of *C. glandulosa* (Table 3). Considering the addition of the inoculant, *Acaulospora* sp.1 *(IV = 0.274. p= 0.01)* and *Funneliformis mosseae (IV = 0.297. p= 0.03)* were selected as indicators of inoculum treatment (Table 4).

Table 3. Occurrence of AMF species in the rhizosphere of six native tree species Atlantic Forest Brazilian.

Family/Species AMF	Plant					
	Pterogyne nitens	***Schinus terebinthifolius***	***Joannesia princeps***	***Tabermaemontana hystrix***	***Colubrina glandulosa***	***Plathymenia reticulata***
Acaulosporaceae						
Acaulospora colombiana Spain & Schenck	x					
Acaulospora delicata (Walker. Pfeiffer & Bloss)		x	x	x	x	x
Acaulospora morrowiae Spain & N.C. Schenck		x				
Acaulospora scrobiculata Trappe		x	x		x	x
Acaulospora spinosa Walker & Trappe						
Acaulospora sp.1	x	x	x			x
Acaulospora sp.2	x	x	x		x	x
Acaulospora sp.3						
Acaulospora walkeri Kramad. & Hedger	x					
Ambisporaceae						
Ambispora sp.1					x	x
Archeosporaceae						
Archeospora sp.1				x		
Archeospora trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker		x			x	x
Gigasporaceae						
Cetraspora pellucida (T.H. Nicolson & N.C. Schenck) Oehl. F.A. Souza & Sieverd.		x			x	x
Diversisporaceae						
Diversispora epigaea (B.A. Daniels & Trappe) C. Walker & A. Schüssler						x
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	x					
Claroideoglomeraceae						
Claroideoglomus etunicatum (W.N.Becker & Gerd.) C.Walker & A.Schüßler	x	x	x	x	x	x
Funneliformis cf. *geosporus* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler			x	x		x
Funneliformis mosseae (T.H.Nicolson & Gerd.) C.Walker & A. Schüßler	x	x	x		x	
Glomus aggregatum (Schenck & Smith) Koske	x	x	x	x	x	x
Glomus sp.1	x		x	x	x	x
Glomus sp.2	x	x	x	x	x	x
Glomus sp.3		x				
Glomus cf. *formosanum* C.G. Wu & Z.C. Chen	x					

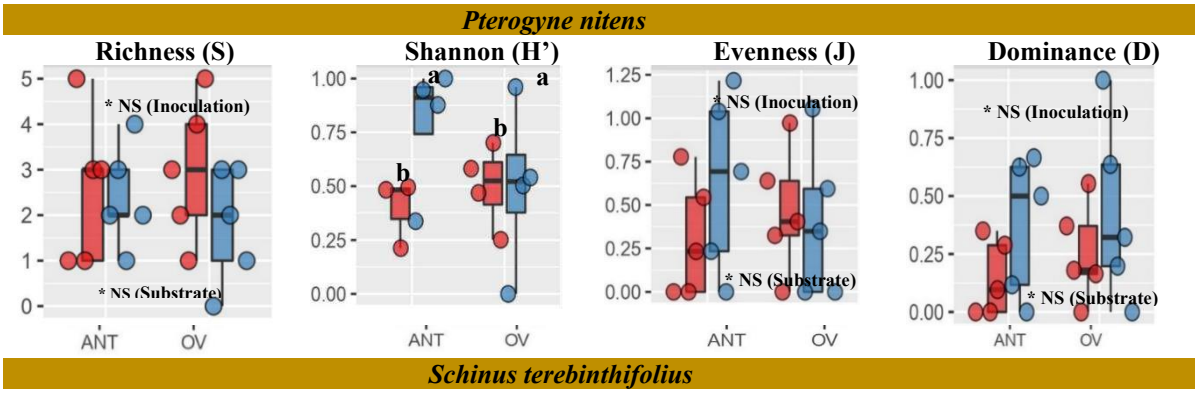
Rhizophagus intraradices (N.C. Schenck & G.S. Sm.) Sieverd. G.A. Silva & Oehl	x	x	x	x	x	x
Septoglomus sp.1	x					
Septoglomus viscosum (T.H. Nicolson) C. Walker					x	
Paraglomeraceae						
Paraglomus sp.1	x	x	x	x		x
Paraglomus albidum (C. Walker & L.H. Rhodes) Oehl. F.A. Souza. G.A. Silva & Sieverd.	x	x	x	x	x	x
Paraglomus occultum (C. Walker) J.B. Morton & D. Redecker	x	x		x	x	x

Table 4. AMF indicator species in the rhizosphere of six native tree species without and with AMF inoculation and cultivated under two substrates (Antuérpia and Ouro Verde).

Species AMF	Plant			Inoculation		
	Species	*IV	*p*	Without/With	*IV	*p*
Acaulospora colombiana Spain & Schenck	-	-	-	-	-	-
Acaulospora delicata (Walker. Pfeiffer & Bloss)	*S. terebinthifolius*	0.536	0.0001	-	-	-
Acaulospora sp.1	-	-	-	**With	0.274	0.01
Acaulospora sp.2	*C. glandulosa* *P. reticulata*	0.346	0.02	-	-	-
Claroideoglomus etunicatum (W. N. Becker & Gerd.) C.Walker & A. Schüßler	*S. terebinthifolius*	0.617	0.0001	-	-	-
Funneliformis mosseae (T.H. Nicolson & Gerd.) C.Walker & A. Schüßler	-	-	-	**With	0.297	0.03
Glomus aggregatum (Schenck & Smith) Koske	-	-	-	-	-	-
Glomus sp.1	*S. terebinthifolius*	0.268	0.04	-	-	-

*IV: indication value
** With: With FMA inoculation
***There were no indicator species for the substrates

The diversity indices of AMF under different inoculation treatments have shown that for the species of *P. nitens* there was an effect of mycorrhizal inoculation only for the Shannon index, with the inoculated treatment promoting the highest diversity value (Figure 1). Regarding the species *C. glandulosa*, the species richness value was substantially higher when inoculated compared to the treatment without inoculation (Figure 1). While, for *S. terebinthifolius*, no effect of inoculation and substrate was found for species richness (Figure 1). However, for the Shannon index and evenness of the AMF species, it was found that the inoculation had an effect on these variables, with the treatment with inoculation which promoted higher values, contrasting with the control without inoculation for species of *S. terebinthifolius* and *C. glandulosa*, except for evenness for the last species, which did not show the effect of inoculation and substrate (Figure 1). Inoculation and substrate did not affect (no significant interactions) any of the diversity indices in *J. princeps* and *P. reticulata* (Figure 1). On the other hand, the substrate influenced the dominance in the species of *T. hystrix* with the highest value observed in the substrate Antuérpia (Figure 1).



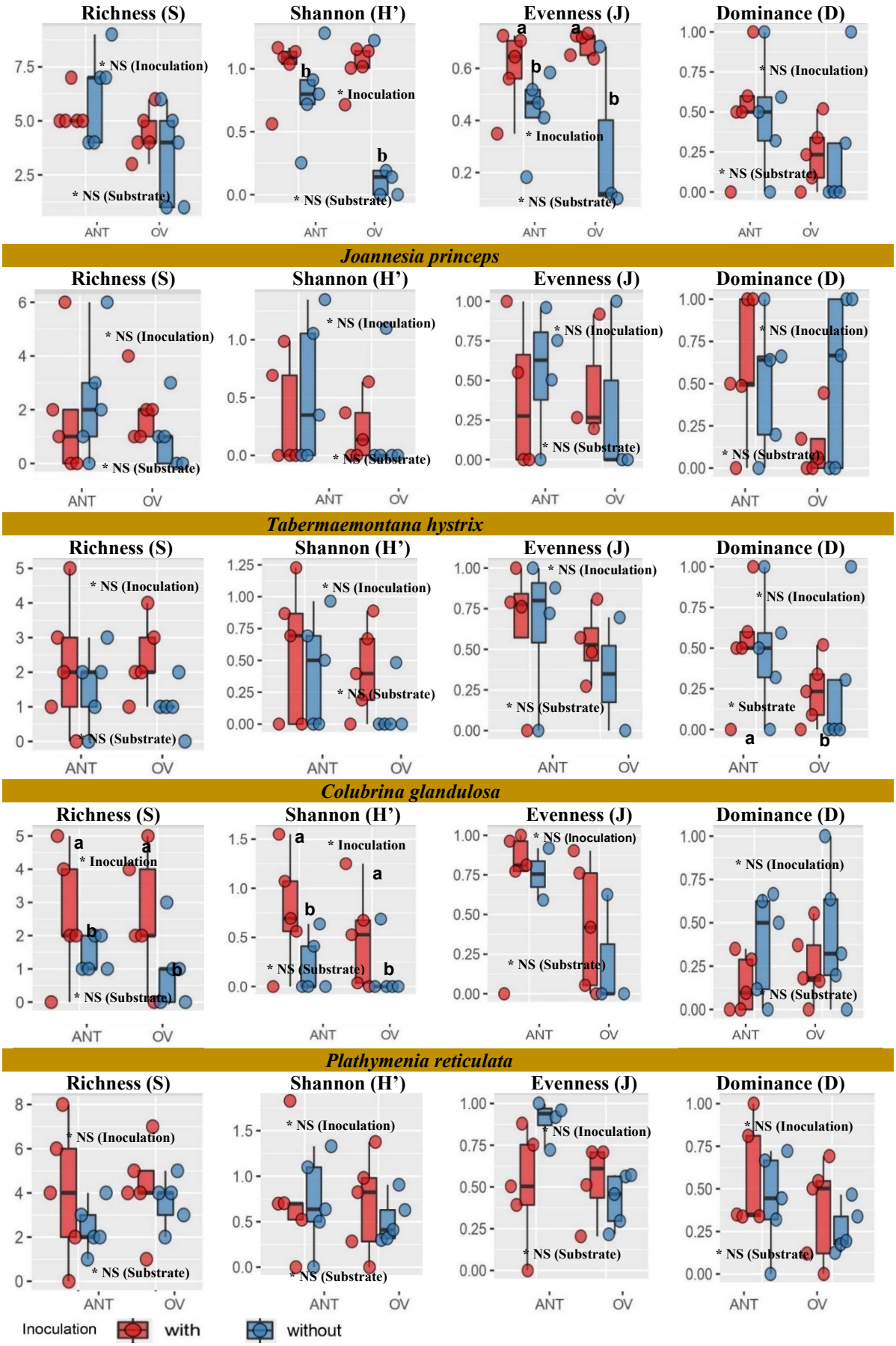
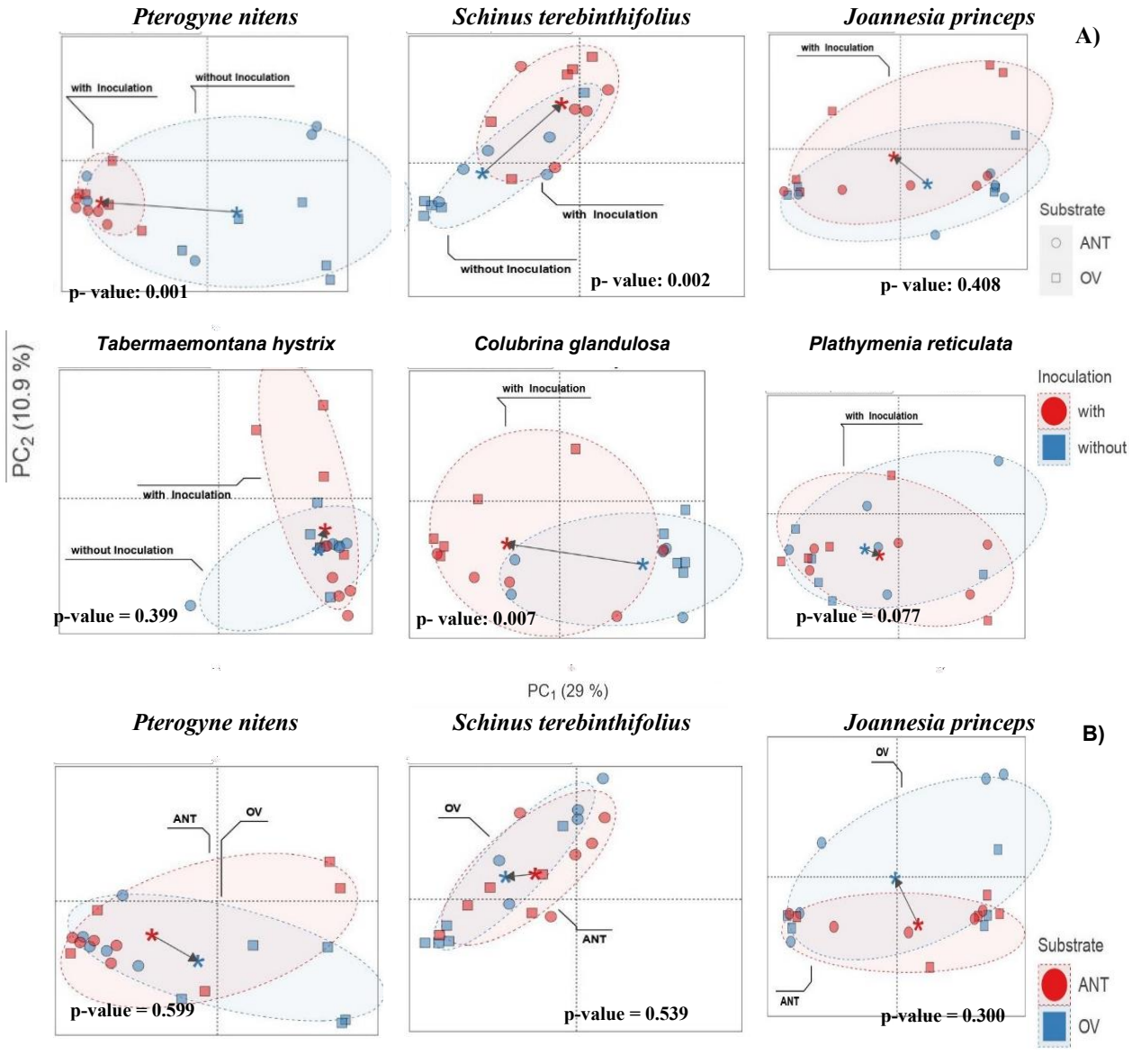

Richness (S)
Shannon (H')
Evenness (J)
Dominance (D)
* NS (Inoculation)
* NS (Substrate)
* Inoculation
* Substrate
a
b
ANT
OV
Joannesia princeps
Tabermaemontana hystrix
Colubrina glandulosa
Plathymenia reticulata
Inoculation
with
without

Figure 1. Richness, diversity, evenness of AMF species in the rhizosphere of six native tree species. at 150 days of cultivation, without and with AMF inoculation and cultivated under two types of substrates (ANT- Antuérpia and OV- Ouro Verde). Different lowercase letters between substrates and inoculation differ by Bonferroni test ($p<0.05$).

Differences were observed in the composition of the AMF community through the addition of the inoculum in *P. nitens* species, *S. terebinthifolius* and *C. glandulosa* but showed no effect for *J. princeps*, *T. hystrix* and *P. reticulata* (Figure 2A). The species of *T. hystrix* was influenced by the type of substrate, while the species *P. nitens*, *S. terebinthifolius*, *J. princeps*, *C. glandulosa* and *P. reticulata* showed no significant differences (Figure 2B). Likewise, mycorrhizal community, depending on the plant species, is clearly separated due to the addition of inoculum and/or cultivation substrate (Figure 2 A and B).



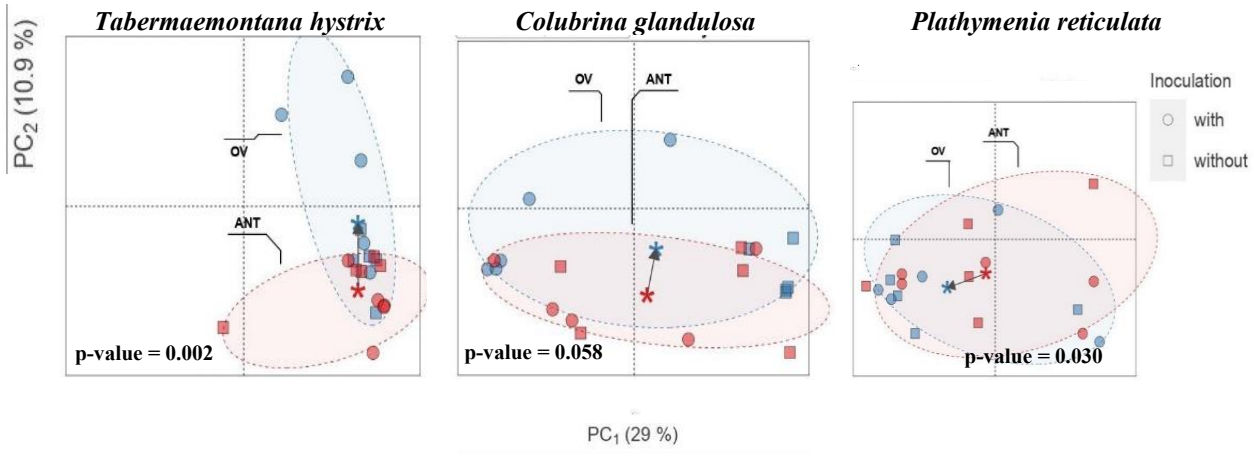


Figure 2. Principal coordinate analysis (PCoA) based on Euclidean distance showing the beta-diversity of AMF and PERMANOVA communities (*p-value*) in the rhizosphere of six native tree species. A) without and with FMA inoculation and B) cultivated under two substrates (OV- Ouro Verde and ANT- Antuérpia).

Seven AMF species were shared between Antuérpia and Ouro Verde substrates for *P. nitens* species (Figure 3 A). Four species were found only in the Ouro Verde substrate (*Acaulospora walkeri*, *Acaulospora* sp.1, *Glomus* cf. *formosanum* and *Paraglomus* sp.1) and five were exclusive to the Antuérpia substrate (*Acaulospora* sp.3. *Funneliformes mosseae*, *Glomus* sp.1, *Scutellospora calospora* and *Septoglomus* sp.1) (Figure 3A). The seven species in common were *Acaulospora delicata*, *Claroideoglomus etunicatum*, *Glomus* sp.2, *Glomus* cf. *aggregatum*, *Paraglomus albidum*, *Paraglomus occultum* and *Rhizophagus* sp.1. For *S. terenbithifolius*, seven species occurred preferentially in the Antuérpia substrate (*Acaulospora colombiana*, *Acaulospora scrobiculata*, *Acaulospora spinosa*, *Acaulospora morrowiae*, *Cetraspora pellucida*, *Glomus* sp. 2 and *Glomus* sp.3) and none were exclusive to Ouro Verde (Figure 3B). However, it was observed that ten species were common between both substrates, being the species*:* *Acaulospora delicata*, *Acaulospora* sp.1, *Archaeospora* sp.1, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus* cf. *aggregatum*, *Paraglomus albidum*, *Paraglomus occultum*, *Paraglomus* sp.1 and *Rhizophagus* sp.1 (Figure 3 B).

As for the species of *J. princeps*, it is observed that five species were shared among the substrates, two of which were exclusive to Ouro Verde (*Acaulospora* sp.1 and *Acaulospora* sp.2) and six to Antuérpia (*Acaulospora scrobiculata, Funneliformis geosporus*, *Paraglomus albidum* and *Rhizophagus* sp. 1) (Figure 3 C). For *T. hystrix* three species were shared between substrates (*Claroideoglomus etunicatum*, *Funneliformes mosseae* and *Glomus* sp.1). three of which occurred exclusively in Ouro Verde (*Acaulospora delicata*, *Archaeospora* sp.1 and

Paraglomus sp.1) and six species (*Glomus* cf. *aggregatum*, *Glomus* sp.2, *Paraglomus albidum*, *Paraglomus occultum* and *Rhizophagus* sp.1) from AMF to Antuérpia (Figure 3D).

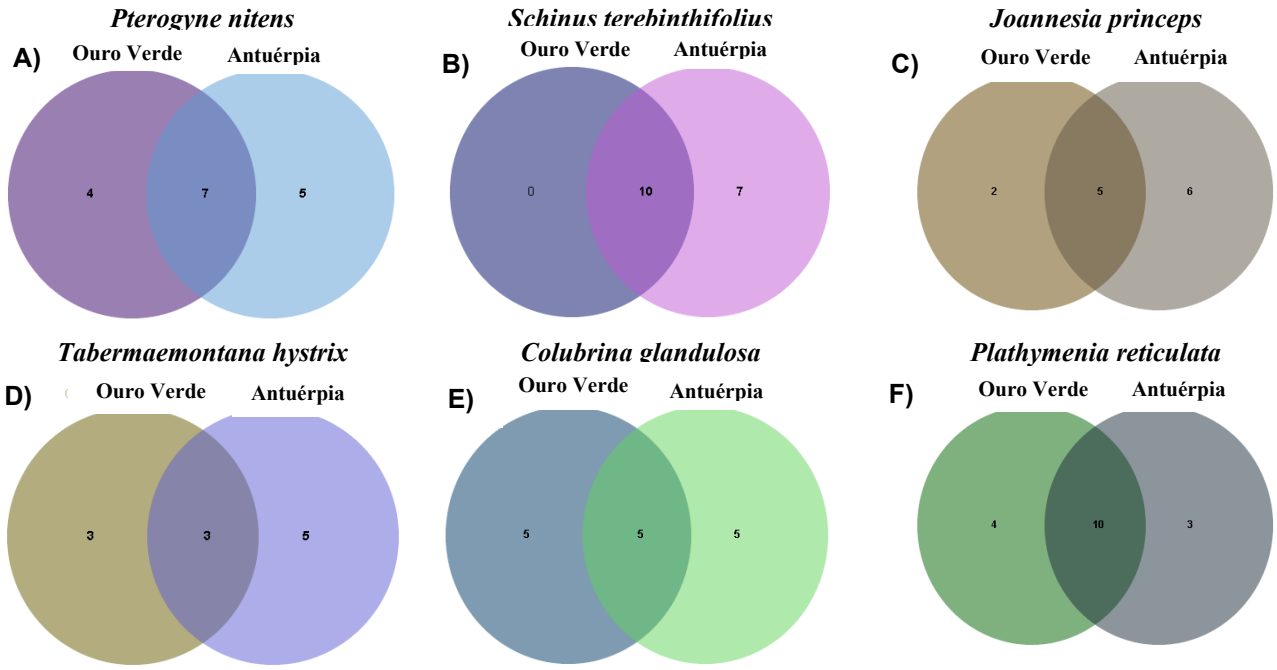


Figure 3. Venn diagram showing the richness of AMF taxa (exclusive and shared) in the rhizosphere of six native tree species cultivated under two substrates, Ouro Verde and Antuérpia.

Discussion

The benefits of using microbial inoculants (AMF) and the influence of using different types of cultivation substrates as an alternative to produce seedlings in forest nurseries that are more resilient to degraded environments have already been reported in other studies (Goetten et al. 2016; Abaurre et al. 2020; Asmelash et al. 2021). However, little is known about the impact of the use of these technologies on the composition of the mycorrhizal community under nursery conditions. All studied plants formed symbiosis with the AMF (Menezes et al. 2022) and there are species belonging to the initial successional group.

In the present study, Antuérpia substrate and mycorrhizal inoculation led to a greater production of glomerospores in seedlings *P. nitens*, *T. hystrix*, *J. princeps* and *C. glandulosa*. These results agree with Pedone-Bonfim et al. (2018) who observed benefits of inoculation in the production of glomerospores in *Mimosa tenuiflora* seedlings inoculated with *G. albida* and a mix of FMA species in a low dose of P, recommending the use of this sustainable technology in the production of this species native to the Caatinga. On the other hand, this greater

sporulation observed in the Antuérpia substrate may be associated with its physical and chemical composition, for example, higher organic carbon content due to the presence of organic matter coming from the chicken litter in relation to the Ouro Verde substrate (Menezes et al. 2022). These results corroborate Gonzaga et al. (2016) who demonstrated that substrates containing avian manure are promising in increasing mycorrhizal sporulation in *Joannesia princeps* and *Hevea brasiliensis* M. Arg. Furthermore, this greater sporulation observed in Antuérpia may also be associated with the presence of a more clayey soil (red-dystrophic latosol). Studying the abundance of spores in seedlings of native forest species from nine nurseries in Ethiopia, Asmelash et al. (2021) argue that mycorrhizal sporulation can be positively influenced by the presence of clay in soils used in substrates as a mixture in the production of seedlings. On the other hand, the addition of AMF-based inoculant in substrates that do not present adequate physical and chemical parameters for the development of the plant root system can lead to changes in the abundance of native AMF infective propagules in the soil, through competition, reducing the presence of AMF in plant roots. bringing negative responses to plant growth (Janousková et al. 2013), which may have happened with Ouro Verde substrate when inoculated. In general, the substrate with the highest water retention capacity, in this case Antuérpia, was the one that promoted the greatest sporulation in four of the six tree species studied. This agrees with Abaurre et al. (2020), who observed a greater number of glomerospores in substrates with greater water buffering capacity, suggesting that this physical characteristic allows fungal hyphae to explore substrates in search of water and stimulate plants to form symbiosis with AMF.

Species of *S. terebinthifolius* is considered an invasive species in Florida and has been extensively studied due to this characteristic (Dawkins and Esiobu, 2017). In Brazil it is considered a native species with pharmaceutical potential, wood and of interest for reforestation of degraded areas (Azevedo et al. 2015). In our study, mycorrhizal inoculation and the cultivation substrate did not promote benefits in the production of glomerospores for this plant, however, we found a high sporulation ranging from 299.50 to 473.75 spores in 20 g of soil compared to other native species. These results are like those found in Florida, where *S. terebinthifolius* species was found to harbor a large number of glomerospores (275 spores/20g) and other beneficial fungal species compared to plant species native to the region (Dawkins and Esiobu, 2017). This is an interesting feature that can help restore the microbiome by introducing this plant species into an area that has suffered some environmental damage, and its use in disturbed areas can be recommended.

The introduction of mycorrhizal inoculant in the Ouro Verde substrate in *P. reticulata* is necessary so that there is more production of glomerospores. Pagano et al. (2009) observed that mycorrhizal inoculation in *P. reticulata* seedlings promoted greater glomerospore production (161 spores/100 g of soil) compared to eucalyptus. However, this greater sporulation may not always mean benefits for the plant since in a previous study by (Menezes et al. 2022) it was found that inoculation in the Ouro Verde substrate promoted lower growth and dry matter production of the part in seedlings of *P. reticulata*. However, it is known that AMF can present functional differences depending on the combination between fungus and plant, bringing other benefits besides plant growth, such as better mineral nutrition (Newsham et al. 1995). Such results may also be related to the high values of E.C (2.88 mS/cm) observed in this substrate, corroborating the work of Silva-Flores et al. (2019) by showing that there is a strong positive correlation between electrical conductivity and number of glomerospores as a result of salt concentration. Furthermore, the use of native inoculants, that is, adapted to these salinity conditions, are much more beneficial than the introduction of exotic inoculants, as seen in seedlings of *Tamarix articulata* (Bencherif et al. 2021).

Acaulospora and Glomus are the most reported genera and present in the rhizosphere of tree seedling species native to Brazil (Pagano et al. 2009; Pagano et al. 2011; Souza et al. 2012; Weber et al. 2021), corroborating the results obtained in this study. Furthermore, most species belonging to these genera are commonly found and adapted to different forest ecosystems (Winagraski et al. 2019; Rodrigues et al. 2021), which demonstrates the high plasticity of these fungi and their potential to be used as inoculants aiming at the production of tree species seedlings.

The composition of the AMF community is influenced by its colonization strategy in different hosts (Onguene et al. 2010; He et al. 2019). So, *A. delicata*, *C. etunicatum* and *Glomus* sp.1 were indicators corresponding to forest species *S. terebinthifolius*. Although we have not quantified all the fungal structures of AMF in plants (Menezes et al. 2020), it was possible to observe a predominance of hyphae, vesicles, and spores in its roots. suggesting that *S. terenbithifolius* has a strong association with fungi of the Glomeraceae and Acaulosporaceae family. These results are similar to those observed by Aziz et al. (1995) and Dawkins and Esiobu (2017), in which they argued that *S. terenbithifolius* plants form preferential association with species of *Glomus* spp., *Acaulospora* spp. and *Rhizophagus* spp. considered generalist fungi. Furthermore, this preferential association with generalist species is interesting for the initial stages of revegetation in a degraded area. *Acaulospora* sp.2 was considered an indicator

species of native plants *C. glandulosa* and *P. reticulata*. Weber et al. (2021) determining the AMF community as soil indicators in native Brazilian tree species in the Acaraú Basin, reported that species of the genus *Acaulospora* are considered environmental indicators for *C. glandulosa* and *Astronium fraxinifolium* plants as observed in our study. Evaluating the effect of double inoculation (rhizobia and AMF) on the occurrence of AMF in *P. reticulata*, Pagano et al. (2009) also observed the presence of species of the Acaulosporaceae family in association with *P. reticulata* after inoculation.

Several other factors may explain this preferential association with certain AMF species by the plant, including changes in root exudates released that are different and modified according to the biotic and abiotic conditions to which these plants are subjected (Marschner, 1995). And our results reinforce the work of Davison et al. (2011) that there may be some host selectivity for certain AMF fungal species.

Inoculation favored the presence of infective propagules of *Acaulospora* sp1 and *Funneliformis mosseae* when native tree seedlings were inoculated, making them an indicator species and such behavior may be related to the life strategy of these fungi, which is in agreement with Chagnon et al. (2013) and Hart and Reader (2002), arguing that members of the Glomeraceae family are the first to colonize plant roots, producing a large volume of intraradicular mycelium, in addition to investing in a large amount of spores, making them more infective compared to other species. Meanwhile, members of the Acaulosporaceae family are considered stress-tolerant fungi, through the production of a more persistent and infective mycelium (Chagnon et al. 2013), which gives this group a more tolerant character against inoculation with a mix of species of AMF. Furthermore, *Funneliformis mosseae* is considered a species with wide global distribution and easy adaptation to different environmental conditions (Rosendal et al. 2009), which suggests that this species is highly competitive against the introduction of the inoculant.

Our initial hypothesis was confirmed that mycorrhizal inoculation can modulate the composition of the AMF community in tropical tree species depending on the type of plant and these results were observed in other studies (Lance et al. 2019; Cornell et al. 2021) and are reinforced by PCoA and PERMANOVA analysis. For example, in *P. nitens* we found that inoculation reduced the diversity of the AMF community. In a field study in Senegal, Thioye et al. (2019) found that the introduction of inoculant (*Rhizophagus irregulares*) in seedlings of *Ziziphus mauritiana* negatively impacts the richness and diversity of the native AMF community, which corroborates our study, since the substrates studied were not sterilized and

already presented a resident AMF community. Furthermore, we work with a consortium of species as an inoculant, and this can cause one of the isolates to settle in space and reduce the presence of native fungi as discussed by (Cornell et al. 2021) negatively affecting the structure and composition of the AMF community. Furthermore, Verbruggen et al. (2013) suggest that the introduction of exotic inoculant can lead to "exogamy depression" through genetic exchanges between exotic and native fungi, which can affect the mutualistic capacity.

In this study, the tree species of *S. terebinthifolius* and *C. glandulosa* had the diversity and richness of AMF species in the substrates increased due to mycorrhizal inoculation, however, this increase in the diversity indices in these two hosts cannot always be considered as a good indicative of better plant performance when inoculated. In a previous study carried out by Menezes et al. (2022) seedlings of *S. terebinthifolius* had their growth reduced because of inoculation, while the species of *C. glandulosa* showed a neutral effect of inoculation with AMF. Such results diverge from most of the studies that argue benefits in plant performance due to the greater richness and diversity of AMF (van der Heidjen et al. 1998; Cornell et al. 2021). However, these native Brazilian tree species are known to harbor a wide diversity of arbuscular mycorrhizae species (Dawkins and Esiobu. 2017; Weber et al. 2021), which may have given these plants other benefits not only linked to plant growth. However, it is worth emphasizing that the species identified in our study were based on spores present in the soil, and the community composition in the roots of these plant species was not evaluated, which may present another result.

The type of cultivation substrate used in seedling production influences the AMF symbiosis in *Samanea saman* seedlings (Abaurre et al. 2020). In the present study, the Antuérpia substrate promoted greater dominance of AMF species in the rhizosphere of *T. hystrix* from the Glomeraceae and Acaulosporaceae family, species that present high plasticity under different biotic and abiotic conditions (Zangaro and Moreira. 2010) and Paraglomeraceae, one species of AMF rarely studied, however, its presence was abundant in field studies with native seedlings of *Z. mauritiana*. These results suggest that the chemical and physical composition of the cultivation substrates, as well as the plant species, selects specific fungal groups for certain environmental conditions. However, there are no reports in the literature evaluating the influence of different types of substrates on the composition of the AMF community, this being the first study.

No reports were found in the literature investigating in depth the effect of the cultivation substrate after mycorrhizal inoculation on the native FMA community. However, the impact of

these technologies on the growth and efficiency of symbiosis in tree species are already reported (Diouf et al. 2019; Abaurre et al. 2021). The high presence of members of the Acaulosporaceae, Glomeraceae and Paraglomeraceae families in the two types of substrates grown independently of the plant host, as observed in the Venn diagram, confirms that these fungal groups have a wide distribution and low specificity in forest species (Thioye et al. 2019), consequently they can adapt to different environmental conditions presented by different substrates. It is interesting to note that *C. etunicatum* was present in all substrates and plants and can be considered a generalist species with high potential to be used and produced as an inoculant by nurserymen, as they can help to restore seedlings transplanted to degraded areas (Winagraski et al. 2019).

However, our results diverge as to the generalist behavior of *A. scrobiculata* recurrently observed in other studies (Winagraski et al. 2019), as a low presence of this species and its specificity to Antuérpia substrate in seedlings of *S. terebinthifolius*, *C. glandulosa*, *J. princeps* and *P. reticulata*. The frequent presence of *A. scrobiculata* in the Antuérpia substrate (pH 6.5) corroborates the fact that this species has a preference for environments, whose pH is more acidic as an observer in Araucaria seedlings produced in nurseries (Vilcatoma-Medina et al. 2018). In addition, the presence of species belonging to the genus *Paraglomus* in all substrates demonstrates that this species has biotechnological potential to be used as an inoculant, and its presence in the rhizosphere of *Cinnamomun camphora* seedlings produced in a nursery in China under two management conditions is also recorded (Zhang et al. 2021).

Furthermore, among five AMF species inoculated into native tree species (*A. scrobiculata*, *C. etunicatum*, *P. albidum*, *Paraglomus* sp. and *P. brasilianum*), only *P. brasilianum* was not detected in the rhizosphere of all plants and in both types of substrates tested after inoculation, suggesting that such species has low capacity to compete with native AMF and/or those present in the inoculant, influencing its functionality. This result corroborates Souza et al. (2012) who also observed the disappearance of some inoculated FMA species, for example, *A. longula*, *C. etunicatum*, *G. glomerulatum* and *G. rubiforme* in the field on seedlings of *Guazuma ulmifolia*.

Conclusion

The present study demonstrates in depth that mycorrhizal inoculation with exotic AMF and the use of different types of cultivation substrates used in forest nurseries modulate the native AMF community in these habitats, mainly by inoculation. Furthermore, plant hosts can

be associate with specific AMF fungal groups as indicator species. The number of glomerospores responds differently to the different native tree species studied and chemical and physical characteristics of cultivation substrate can affect AMF sporulation.

The most frequent genera in the rhizosphere of the six native species are *Acaulospora*, *Glomus* and *Paraglomus* regardless of the substrate. Thus, it is suggested that further studies could be carried out to understand how the interaction between introduced and native AMF strains occurs and whether such changes compromise the performance and quality of seedlings in the nursery.

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5. Chapter 3: Written according to Forest Ecology and Management

Arbuscular mycorrhizal fungi inoculation during seedling formation and growth physiological responses during establishment of native tree species in the field

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Abstract

The lack of arbuscular mycorrhizal fungi (AMF) propagules in degraded soils may contribute to the failure of forest restoration. Thus, it is necessary to incorporate AMF propagules in the substrate of cultivation during seedling formation to be introduced in the field. Moreover, the functional and photosynthetic responses of plants may be modified as a function of environmental factors and substrate characteristics, determining plant performance. Seedlings of six Atlantic Forest native tree species (*Pterogyne nitens, Schinus terebinthifolius*, *Joannesia princeps*, *Tabermaemontana hystrix* e *Colubrina glandulosa*) with 150 days-old, produced in two types of substrates (Antuérpia and Ouro Verde), one inoculated with AMF (Antuérpia) and the other without inoculation (Ouro Verde), were transplanted in a degraded pasture area. After 6 and 12 months I the field the following functional traits were evaluated: height, stem diameter (SD), Relative volume trunk (RVT), relative growth rate (RGR), specific leaf area (SLA) and photosynthetic characteristics (Fv/Fm, Total chlorophyll, *a* and *b*). As expected, all species studied showed greater growth, SD and RVT as a function of age (rainy season). For *P. nitens* and *J. princeps* higher SLA was observed in the dry period (6 months), while at 12 months *T. hystrix* showed higher SLA and when cultivated in Antuérpia substrate, and showed higher Fv/Fm ratio. In addition, there was a strong seasonal effect on physiological responses, in which a greater functioning of PSII (Fv/Fm) was observed in the rainy season for *P. reticulata*. As for photosynthetic pigments, only *C. glandulosa* and *P. reticulata* were affected by the seasonality, with higher total chlorophyll *a* and total in the dry season in the first species and chlorophyll *a* and Fv/Fm for the last plant in the rainy season. Our study reinforces once again that

mycorrhizal sporulation is influenced by seasonality with the highest number of glomerospores observed for all tree species at 6 months (dry period). In general, we found a neutral effect of the introduction of mycorrhizal propagules in the substrate used for seedlings production. On the other hand, we found a strong seasonal effect in all traits studied, contradicting the idea that low water availability during the dry season reduces photosynthetic efficiency and mycorrhizal activity, showing the necessity of more studies of plant-AMF and physiological aspects of Atlantic Forest native trees species.

Keywords: Chlorophyll fluorescence, Revegetation, Seasonality, Native tree species, substrate, AMF, Atlantic Forest

Introduction

According to the United Nations Framework Convention on Climate Change (2016) Brazil must restore 12 million hectares of forested areas by 2030 based on the Paris agreement. However, in the long term, it is estimated that this forest restoration target will reach more than US$ 8.9–15.6 billion, presenting a high cost (Brancalion et al. 2019), as a result, alternatives have been sought in order to mitigate these costs. However, to be successful in recovering a heavily degraded environment it is necessary to make the soil biologically active. Among the strategies, the introduction of seedlings associated with of beneficial soil microorganisms can be the initial step in the restoration process, mainly aiming at reducing costs with the use of mineral fertilizers and a more sustainable agriculture (Pascual et al. 2018). Thus, it is of great importance to recover areas in order to reverse this scenario through the reintroduction of native forest seedlings with the presence of beneficial microorganisms (Asmelash et al. 2021). However, commercially sold substrates are inert, that is, they lack the presence of these microorganisms, such as arbuscular mycorrhizal fungi (AMF) that can reduce the application of mineral fertilizers and improve plant development.

AMF are known to be benefit to the growth of native tree species in nurseries (Pedone-Bonfim et al. 2018; Abaurre et al. 2021) and, mainly, for exploring the soil through their hyphae in search of more limiting nutrients, such as phosphorus (P) (Smith and Read, 2008; Smith and Smith, 2011). Furthermore, these fungi have been key to the process of revegetation and forest recovery (Asmelash et al. 2016). Thus, evaluating and selecting substrates with the most promising physical and chemical characteristics in favoring and potentiating the role of AMF in seedling production is important (Abaurre et al. 2020 and 2021). However, under field

conditions, little is known about the effect of these inoculated symbionts in different substrates on the establishment and physiological responses of plants over time, since seasonality has a strong effect on plant fitness (Afonso et al. 2020) affecting their physiological functions. Among plant species used in this study, five have already been reported in the literature (*Schinus terebinthifolius*, *Colubrina glandulosa*, *Pterogyne nitens*, *Plathymenia reticulata* and *Joanna princeps*) as to be potential for using in revegetation programs in degraded areas in the Atlantic Forest and Brazilian Cerrado (Gomes et al. 2018; Moraes Júnior et al. 2019). However, some species may have their photosynthetic apparatus compromised due to seasonal fluctuations and the growth substrate (Dos Anjos et al. 2015; Afonso et al. 2020). For example, *S. terebinthifolius* is considered a species of high photosynthetic plasticity capable of display functional foliar adjustments and tolerate stressful environments, while *J. princeps* showed medium plasticity (Dos Anjos et al. 2015). In addition, when seedlings are transplanted to environments with high sun exposure, which occurs in areas that have been degraded, the plants may undergo a process called photoinhibition (Craven et al. 2011), which may reflect on the performance and establishment of seedlings in the field.

In addition to the seasonal effects, the physical and chemical composition of the cultivation substrates and the inoculation with AMF propagules can influence the production of photosynthetic pigments due to the availability of water and nutrients. In a nursery, Afonso et al. (2020) producing seedlings of *Tabermaemontana catharinensis* did not observe the influence of substrate composition on the production of chlorophyll *a*, *b* and total. On the other hand, Mota et al. (2016) in *Eugenia dysenterica* seedlings observed that the presence of organic residues in the substrate composition favors the Fv/Fm ratio, electron transport rate and plant growth. Regarding the effect of mycorrhizal inoculation in the cultivation substrate, El-Khateeb et al. (2011) producing *Acacia saligna* seedlings found that the content of chlorophyll *a* and *b* increased in treatments inoculated with AMF.

Using meta-analysis, Neuenkamp et al. (2019) concluded that AMF can facilitate establishment, plant growth and forest restoration in the field. However, the presence of AMF propagules in the seedlings growing substrate when taken to the field can lead to positive, neutral or negative changes in resident microbial communities, reflecting on the performance of introduced seedlings (Mummey et al. 2009; Lance et al. 2019) and composition from the plant community (van der Heidjen et al. 1998). For example, Souza et al. (2012) observed that the mycorrhizal inoculation of seedlings of *Guazuma ulmifolia* and *Tabebuia roseo-alba* transplanted to the field did not change the number of glomerospores, however, it modulates

the composition of the resident AMF community, and was beneficial for the establishment of the two tree species.

In addition to inoculation, seasonal fluctuations over time are known to influence mycorrhizal propagules production and root phenological stage (Guadarrama and Álvarez-Sánchez, 1999). Thus, the quantification of AMF spores has been one of the alternatives suggested and used to determine the mycorrhizal dynamics under adverse conditions, as it is a resistance structure (Abbout and Robson, 1991). Over time, spores production can be modulated as a function of the seasons, and consequently can be used as an indicator of seasonal changes, as drought is known to increase sporulation (Pereira et al. 2018; Vieira Júnior et al. 2020), with soil moisture also being an important predictor (Moebius-Clune et al. 2013) between seasons. Thus, the hypothesis of this work was that: I- the presence of infective AMF propagules in the cultivation substrate favors the growth and establishment of six native tree species in a degraded pasture area over time, II - the substrate of cultivation containing AMF promotes improvements in functional (SLA, RGR) and photosynthetic (Fv/Fm and chlorophyll content) traits regardless of the season, and finally III – the dry and rainy seasons can influence the photosynthetic adjustments of plants and the mycorrhizal dynamics in the field. Thus, the objective of this study was to evaluate, in the field, the effects of the introduction of two substrates, one containing AMF propagules (Antuérpia) and another without inoculation (Ouro Verde) in the establishment of seedlings used for revegetation of degraded pasture area, as well as to observe the effects of the dry and rainy seasons and the possible changes that these factors can cause on plant ecophysiology and mycorrhizal dynamics in the field.

Material and methods

Study area

The study was carried out in a degraded pasture area, located in the municipality of Viçosa, Zona da Mata Mineira (Southeast Brazil) (-20.8092415S, -42.835078W). The air temperature and average annual precipitation in the region is 20.4 ºC and 1251 mm/year, ranging from 12 mm in July (dry) to 252 mm in December (https://pt.climate-data.org/america-do-sul/brasil/minas-gerais/vicosa-25021/). The climate of the region is Cwa according to the Köppen classification and data on precipitation, temperature and solar radiation for the periods collected are shown in figure 1. For the present study, six native plant species of the Atlantic Forest Biome were chosen, based on a list made available by the Renova Foundation. The selected native plant species were: *Pterogyne nitens* Tul. (Fabaceae), *Schinus terebinthifolius*

Raddi. (Anacardiaceae), *Joannesia princeps* Vell. LC. (Euphorbiaceae), *Tabermaemontana hystrix* Steud (Apocynaceae), *Colubrina glandulosa* Perkins (Rhamnaceae) e *Plathymenia reticulata* Benth. (Fabaceae).

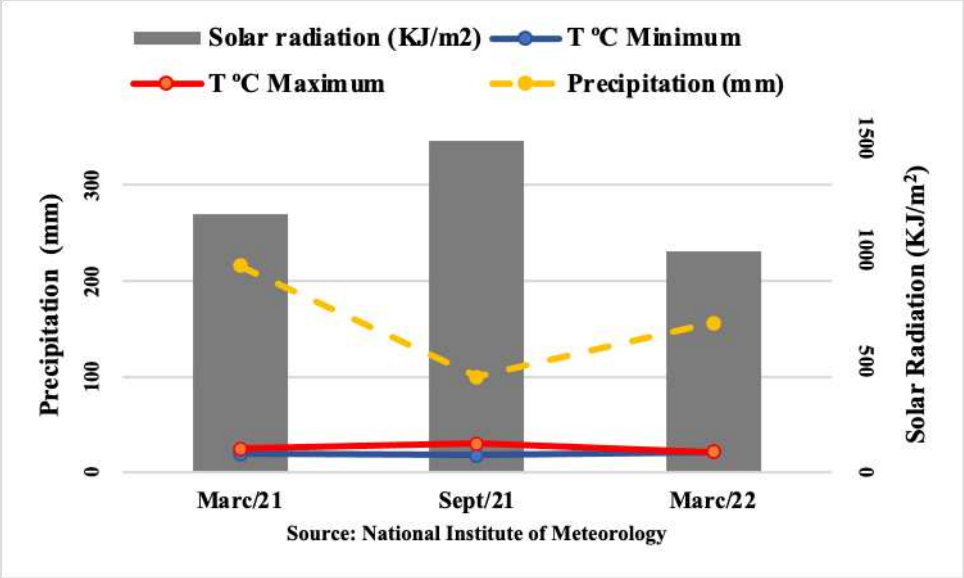

Figure 1. Average rainfall, temperatures (minimum and maximum) and radiation from March 2020 to March 2021, Viçosa-MG.

Seedling preparation

The seedlings of six native species were produced in the commercial nursery Ouro Verde (https://www.facebook.com/ouroverde.viveiro/), located in the city of Belo Oriente, Minas Gerais, Southeast of Brazil (19°11' 39.7"S 42°24'50.9"W) in September 2019. Seeds were germinated in sand and the uniform seedlings that presented the first pair of true leaves (about one month) were selected, and then transplanted to the tubes containing the 280 mL of substrates Ouro Verde (without inoculation) or Antuérpia, inoculated with a mix of AMF species (*Acaulospora scrobiculata* Trappe, *Paraglomus albidum* cf. C. Walker & L.H. Rhodes, *Paraglomus brasilianum* (Spain & J. Miranda) J.B. Morton & D. Redecker, *Paraglomus* sp., *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler.). The substrate Antuérpia was produced in the Antuérpia nursery in Viçosa-MG, and was inoculated with a mix of AMF species, while the substrate Ouro Verde is used by the commercial nursery Ouro Verde in Belo Oriente-MG, however, it was not inoculated. The seedlings were cultivated in a commercial nursery in full sun and received daily irrigation by microsprinkler for 7 min every 1 h, varying according to the climatic conditions of the day, starting at 8 am and ending at 4 pm. Descriptions of the methods and detailed information on seedling production and preparation of the AMF inoculant are present in chapters 1 and 2 of the thesis. The seedlings

were kept in a nursery for 150 days, and then they were transplanted to the field, which corresponded to a degraded and abandoned pasture area.

Descriptions of the area and experimental design

The experiment was conducted in a degraded and abandoned pasture area and the experimental area was fallow and without any agricultural practices.

In 2020, before the introduction of native tree species, soil was collected for chemical analyses. Three fertilizations were carried out as general recommendations for native trees: one before planting the seedlings in the field (end of March 2020), in which 2 L of goat manure plus 140 g of NPK 6-30-6 per hole and two topdressing fertilizations were applied, one in May of the same year and another in January 2021, with 80 g of NKP 20-05-20 being applied per plant. The experimental area was initially irrigated every 15 days in the first two months after seedlings were implanted in the field. We decided to irrigate the initial months due to the implantation coinciding with the end of the rainy season and the beginning of the dry season.

The design was in randomized blocks in plots subdivided in time, being the substrates (Ouro Verde -Without inoculation and Antuérpia-With inoculation) considered as plots, and the time periods (6 and 12 months - dry and rainy) as subplots, with five repetitions. The seedlings were planted in a pasture area to be recovered, adopting a spacing of 2.5 x 2 m. The distribution of plants within each subplot was performed randomly, with 1 plant of each of the six native tree species being allocated under two substrates, totaling 60 plants. The plot area was 260 m² (10 x 26 m), each one being separated from the neighboring plot by a distance of 30 m, in order to isolate the areas. At the time of transplanting, the seedlings height, stem diameter, mycorrhizal colonization of each seedling was measured (Table 1), and the experimental area had an average of 568 spores/50g of soil glomerospores.

Table 1. Height, stem diameter, mycorrhizal colonization and number of glomerospores before transplanting the seedlings.

Espécies/common name	**Height (cm)**		**Stem diameter (mm)**		**Mycorrhizal Colonization (%)**	
	Ouro Verde	**Antuérpia**	**Ouro Verde**	**Antuérpia**	**Ouro Verde**	**Antuérpia**
P. nitens (Amendoim-bravo)	8.58	9.96	3.14	3.11	95.6	23.8
S. terebinthifolius (Aroeira)	34.46	33.76	7.06	7.69	85.8	95.8
J. princeps (Boleira)	39.74	38.12	12.25	14.95	29.6	86.4
T. hystrix (Leiteiro-jasmin)	8.40	12.88	3.55	3.33	8.0	40.2
C. glandulosa (Saguaraji)	14.84	15.84	4.68	5.57	50.8	85.8
P. reticulata (Vinhático)	50.84	40.20	7.18	6.20	37.0	51.6

Average of 5 plants

Analysis of soil chemical properties

Soil chemical analyzes were carried out in a commercial laboratory (Laboratório de Análise do Solo Viçosa, in Viçosa, MG, Brazil, using the methodology described by Defilipo and Ribeiro, 1997). Chemical characterization was performed for each period collected (Table 2). To determine the content of P and K they were extracted with Mehlich-1 extractor and Ca^{2+} and Mg^{2+} with KCl 1.0 N. The pH of the soil was determined in a soil solution: water (1:2.5 v/v), and the exchange capacity cation (CEC (t), CEC (T)) was calculated by the sum of bases and potential acidity.

Soil sampling

Sampling was carried out at the end of March and September 2021 and March 2022, on three occasions: before the introduction of seedlings, in order to characterize the experimental area, at 6 months (dry) and 12 months (rainy) after the introduction of the seedlings. seedlings in the field. Soil samples were collected from the rhizosphere of each tree species and substrates at a depth of 0-10 cm.

Morphophysiological characteristics of plant

Measurements of height, stem diameter, relative growth rate, relative volume trunk and specific leaf area were taken at each sampling period (6 and 12 months – dry and rainy seasons). To determine the height and diameter of the stem, a millimeter ruler and digital caliper were used. The trunk volume was determined for each species studied using the formula provided below for further determination of the trunk volume rate (RVT). The relative growth rate (RGR) and relative volume trunk was calculated for each species, using the following equation:

$$\text{a) } RGR = \frac{\ln(\text{Height}_F) - \ln(\text{Height}_0)}{t_f - t_0}$$

$$\text{b) i: } = F*\pi*(D/10)^2*H \text{ (Volume trunk)}$$

$$\text{ii: } RVT = \frac{\ln(\text{Volume}_F) - \ln(\text{Volume}_0)}{t_f - t_0}$$

a) Where, Height_F: final height; Height_0: initial height and: t_f = final time and t_0 = initial time (in months)

b) Where, F: 0.35 (form factor); π: 3.1415; D: Stem diameter; H: heigh

Volume_F: final volume; Volume_0: initial volume and: t_f = final time and t_0 = initial time (in months)

To determine the specific leaf area (SLA), young, pathogen-free and fully expanded leaves were selected (Pérez-Harguindeguy et al. 2013). Fresh leaves were photographed to obtain computer images, and then the total leaf area (TLA) was determined using ImageJ software (Rasband, 2009). After the determination of the images to obtain the total leaf area, the leaves were packed in paper bags and placed to dry in a forced circulation oven at 65 ºC until their leaf mass was stabilized, later the leaves were weighed to obtain the total dry biomass (TDB). Soon after, the SLA was determined by dividing the total leaf area by the leaf dry mass. Measurements of the maximum quantum efficiency of PSII (Fv/Fm) were determined after the leaves had been dark adapted for 30 min, using the fluorometers MINI-PAM (Walz, Effeltrich, Germany) and FluorPEN FP 110 (Photon Systems Instruments, Drasov, Czech). Measurements were made in dark-acclimated leaves (30 min minimum) after exposure to 1 s saturating light pulses according to the method described by Maxwell and Johnson (2000). The chlorophyll content (*a*, *b* and total) was determined using an electronic meter (ClorofiLOG®, Falker Automação Agrícola Ltda., Porto Alegre, Brazil) and expressed as a Falker Chlorophyll Index (IFC) (Falker, 2008).

Extraction and counting of glomerospores

50 g of soil from each sample was used to extract the glomerospores using the wet sieving technique, followed by centrifugation in water and 40 % sucrose (Gerdemann and Nicolson 1963; modified Jenkins 1964), placed on glass plates and counted under stereomicroscope (40x).

Statistical analysis

Univariate analyzes were performed using the SISVAR statistical program. Soil chemical data, morphophysiological characteristics and number of glomerospores were submitted to ANOVA and means compared by Student's t test ($p<0.05$). Principal Coordinate Analysis (PCA) and PERMANOVA were performed to analyze the effect of time period (6 and 12 months - dry and rainy) on soil chemical properties, growth and physiological variables in the native tree species studied. For this, the software PAST (Paleontological Statistics) version 4.03 (Hammer et al. 2001) was used.

Results

Soil chemical properties in the rhizosphere of six native tree species in the field as a function of the collection period and cultivation substrate

In the present study, it was observed that soil chemical properties were influenced by time ($p<0.05$) (Table 2). The pH, P, Ca^{2+}, Mg^{2+}, SB, CEC(t) and CEC(T) values showed higher values at six months (dry period) in all six native tree species studied, with the exception of Mg^{2+} which did not show differences for *J. princeps* and *T. hystrix*, and CEC (T) for S. *terebinthifolius* and *T. hystrix* species.

Table 2. Soil chemical characterization considering the substrates and the periods of 6 and 12 months (dry and rainy) after the establishment of six native tree species in the field.

	pH	P	K	Ca	Mg	Al	H+Al	SB	CEC(t)	CEC(T)
	H_2O		mg/dm^{-3}				cmol $_c$/dm^{-3}			
Pterogyne nitens										
Substrates										
Ouro Verde	5.6a	80.57a	483.00a	3.54a	2.58a	0.04a	5.44a	7.76a	7.77a	13.21a
Antuérpia	5.9a	40.40a	365.80a	3.95a	2.39a	0.01a	4.55a	6.70a	6.74a	11.03a
Time										
6 month (dry)	5.6a	93.55a	504.00a	4.29b	3.49a	0.02a	4.49a	9.16a	9.18a	13.42a
12 month (rainy)	5.9a	27.42b	244.80b	3.19a	1.48b	0.03a	5.51a	5.31b	5.34b	10.82b
Schinus terebinthifolius										
Substrates										
Ouro Verde	5.3a	39.18a	299.40a	2.29a	1.36b	0.04a	5.58a	4.42a	4.60a	9.99a
Antuérpia	5.6a	37.66a	321.40a	2.93a	1.78a	0.18a	5.11a	5.54a	5.56a	10.65a
Time										
6 month (dry)	5.5a	65.79a	382.00a	3.19a	1.95a	0.04a	5.29a	6.12a	6.14a	10.41a
12 month (rainy)	5.3a	11.05b	238.80b	2.03b	1.19b	0.18a	6.40a	3.84b	4.02b	10.24a
Joannesia princeps										
Substrates										
Ouro Verde	5.4a	67.26a	343.00a	2.97a	1.70a	0.07a	5.40a	5.55a	5.62a	10.96a
Antuérpia	5.5a	88.02a	420.70a	3.17a	1.95a	0.05a	5.90a	6.20a	6.25a	12.11a
Time										
6 month (dry)	5.6a	134.86a	541.00a	3.88a	2.48a	0.00a	5.98a	7.73a	7.73a	12.72a
12 month (rainy)	5.4a	20.42b	222.70b	2.26b	1.19a	0.01a	6.34a	4.02b	4.13b	10.35b
Tabermaemontana hystrix										
Substrates										
Ouro Verde	5.7a	51.21a	344.90a	3.34a	1.58a	0.00a	4.42a	5.81a	5.86a	10.23a
Antuérpia	5.7a	46.81a	324.60a	3.59a	1.77a	0.01a	4.85a	6.20a	6.23a	22.31a
Time										
6 month (dry)	5.6a	75.74a	449.00a	3.63a	1.83a	0.00a	4.89a	6.61a	6.70a	11.80a
12 month (rainy)	6.0a	22.28b	220.50b	3.30b	1.52a	0.01a	5.38a	5.39b	5.39b	10.77a
Colubrina glandulosa										
Substrates										
Ouro Verde	5.7a	41.92a	460.50a	3.46a	1.79a	0.00a	4.19a	6.44a	6.44a	10.65a
Antuérpia	5.7a	62.35a	452.00a	3.12a	1.93a	0.00a	4.79a	5.95a	5.98a	10.73a
Time										
6 month (dry)	5.6a	86.55a	528.00a	3.76a	2.36a	0.01a	4.19a	7.47a	7.50a	11.56a
12 month (rainy)	5.7a	17.62b	284.50b	2.83b	1.36b	0.01a	4.88a	4.91b	4.91b	9.82b
Plathymenia reticulata										
Substrates										
Ouro Verde	5.5a	38.07a	398.80a	3.25a	2.00a	0.00a	4.45a	7.14a	7.15a	11.43a
Antuérpia	5.9a	31.80a	413.00a	4.48a	2.49a	0.00a	4.49a	7.17a	7.20a	11.72a
Time										
6 month (dry)	5.7a	96.40a	534.00a	4.32a	3.04a	0.00a	4.19a	8.73a	8.74a	12.82a
12 month (rainy)	5.7a	43.47b	277.80b	3.41b	1.45b	0.00a	4.75a	5.57b	5.60b	10.33b

Values followed by different letters in the column, differ from each other by the student t test 0.05 %. Extractors used: P, K = Mehlich1; Ca^{2+}, Mg^{2+} e Al^{3+} = KCl 1 mol. L^{-1}; H + Al = Calcium acetate 0.5 mol. L^{-1}; SB = Sum of exchangeable bases; CEC (t) = Effective cation exchange capacity; CEC (T) = Cation exchange capacity at pH 7.0.

Morphophysiological variables are modulated as a function of the period of evaluation

As for the morphophysiological variables of the six native tree species studied, it was observed that the factors tested (substrates x time) were manifested in isolation, with time (table 1) being the main factor to modulate the growth and physiological parameters (table 3). When performing the splitting of the substrate in each period, we found greater height in the substrate Ouro Verde at 12 months in relation to Antuérpia for *P. nitens*. However, when we broke down the times for each type of substrate, as expected all tree species studied had greater height and stem diameter at 12 months compared to 6 months after seedling establishment in the field (Table 3). When evaluating the relative growth rate (RGR) we did not observe differences between the times and the substrates within each time for all plant species evaluated ($p > 0.05$). On the other hand, the trunk volume rate (RVT) increased over time in all tree species.

The total leaf area (TLA) and dry biomass of the aerial part (SDB) of all tree species studied increased at 12 months (rainy season), with the exception of *T. hystrix*, which did not show significant differences, while the specific leaf area (SLA) also changed between plant development times, with the highest averages observed at 6 months for the species of *P. nitens* and *J. princeps* in relation to at 12 months (Table 3). However, for *C. glandulosa* the substrate Antuérpia at 12 months showed higher SLA compared to Ouro Verde and *T. hystrix* only increase SLA at 12 months.

When evaluating the fluorescence parameters for the six species in the two analyzed periods (Table 4), we observed higher Fv/Fm for *P. reticulata* in the period of 12 months, while *T. hystrix*, the chlorophyll fluorescence was influenced by the substrate, with Antuérpia promoting the highest Fv/Fm ratio in relation to the Ouro Verde substrate. However, no significant variations were observed in the Fv/Fm ratios for the other tree species (Table 4). As for the photosynthetic pigments (chlorophyll *a*, *b* and total), we observed that the values of chlorophyll *a* and total are higher at 6 months (dry season) compared to 12 months (rainy season) for *C. glandulosa* and *P. reticulata*, which showed a significant effect only for chlorophyll *a*. However, no effect at the significance level was observed for the other plant species (Table 4).

Table 3. Height, stem diameter, Shoot dry biomass (SDB), Relative volume trunk (RVT), relative growth rate (RGR), total leaf area (TLA) and specific leaf area (SLA) of six native tree seedlings in the field that were cultivated in two types of substrates, one inoculated with AMF and the other not inoculated in periods of 6 and 12 months (dry and rainy) in the field.

	Pterogyne nitens											
	Height (cm)			**Stem diameter (mm)**			**SDB (g^{-1})**			**RVT (cm^3 cm^3)**		
			Average			**Average**			**Average**			**Average**
	6 month (dry)	**12 month (Rainy)**		**6 month (dry)**	**12 month (Rainy)**		**6 month (dry)**	**12 month (Rainy)**		**6 month (dry)**	**12 month (Rainy)**	
Substrates												
Ouro Verde	23.60aB	167.60aA	95.60	4.36aA	16.37aA	10.37	0.69aB	3.62aA	2.16	1.55aB	6.10aA	3.83
Antuérpia	24.20aB	107.60bA	65.90	4.64aA	12.73aA	8.69	0.70aB	2.09aA	1.34	1.69aB	4.96aA	3.33
Average	23.90	137.60		4.50	14.55		0.64	2.86		1.62	5.53	
	RGR (cm cm^2 day^{-1})			**TLA (cm^2)**			**SLA (cm^2g^{-1})**					
			Average			**Average**			**Average**			
	6 month (dry)	**12 month (Rainy)**		**6 month (dry)**	**12 month (Rainy)**		**6 month (dry)**	**12 month (rainy)**				
Substrates												
Ouro Verde	0.208aA	0.272aA	0.242	82.00	185.73	133.86	127.54	51.16	89.35			
Antuérpia	0.276aA	0.244aA	0.259	98.83	142.88	120.86	168.74	71.36	120.05			
Average	0.240	0.260		90.41b	164.31a		148.14a	61.26b				
	Schinus terebinthifolius											
	Height (cm)			**Stem diameter (mm)**			**SDB (g^{-1})**			**RVT (cm^3 cm^3)**		
			Average			**Average**			**Average**			**Average**
	6 month (dry)	**12 month (Rainy)**		**6 month (dry)**	**12 month (rainy)**		**6 month (dry)**	**12 month (Rainy)**		**6 month (dry)**	**12 month (Rainy)**	
Substrates												
Ouro Verde	119.40aB	236.20aA	177.80	22.76aB	50.52aA	36.64	1.47aA	0.81aA	1.14	6.46aB	8.65aA	7.56
Antuérpia	126.60aB	245.00aA	185.80	23.51aB	50.30aA	36.90	1.12aA	1.08aA	1.09	6.59aB	8.75aA	7.67
Average	123.00	240.60		23.14	50.41		1.29	0.94		6.53	8.70	
	RGR (cm cm^2 day^{-1})			**TLA (cm^2)**			**SLA (cm^2 g^{-1})**					
			Average			**Average**			**Average**			
	6 month (dry)	**12 month (Rainy)**		**6 month (dry)**	**12 month (rainy)**		**6 month (dry)**	**12 month (Rainy)**				
Substrates												
Ouro Verde	0.325aA	0.330aA	0.323	87.10aA	57.44aA	72.27	67.15aA	74.79aA	70.97			
Antuérpia	0.305aA	0.340aA	0.327	82.81aA	71.92aA	76.91	76.89aA	72.04aA	74.47			
Average	0.315	0.335		84.95	64.23		72.02	73.41				
	Joannesia princeps											

	Height (cm)			Stem diameter (mm)			SDB (g^{-1})			RVT (cm^3 cm^3)		
			Average			Average			Average			Average
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)	
Substratos												
Ouro Verde	75.00aB	205.20aA	140.10	16.01aB	51.40aA	33.71	1.00aB	9.30aA	5.15	5.28aB	8.59aA	6.93
Antuérpia	72.40aB	227.80aA	150.10	22.48aB	58.42aA	40.45	0.99aB	7.56aA	4.27	5.93aB	8.97aA	7.45
Average	73.70b	216.50a		19.24	54.91		1.00	8.42		5.61	8.78	
	RGR (cm cm^2 day^{-1})			TLA (cm^2)			SLA (cm^2g^{-1})					
			Average			Average			Average			
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)				
Substratos												
Ouro Verde	0.278aA	0.270aA	0.274	151.44aB	784.87aA	468.15	159.23aA	84.50aB	116.49			
Antuérpia	0.266aA	0.268aA	0.267	151.43aB	536.79bA	344.11	156.81aA	76.18aB	121.87			
Average	0.272	0.269		151.43b	660.83a		158.02	80.43				
	Tabermaemontana hystrix											
	Height (cm)			Stem diameter (mm)			SDB (g^{-1})			RVT (cm^3 cm^3)		
			Average			Average			Average			Average
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)	
Substrates												
Ouro Verde	37.60aB	127.60aA	82.60	6.13aB	16.30aA	11.22	0.32aA	0.28aA	0.30	2.59aB	5.57aA	4.08
Antuérpia	42.00aB	133.60aA	87.80	8.47aB	19.53aA	14.00	0.32aA	0.40aA	0.36	3.47aB	6.15aA	4.81
Average	39.80	130.60		7.30	17.92		0.32	0.34		3.03	5.86	
	RGR (cm cm^2 day^{-1})			TLA (cm^2)			SLA (cm^2 g^{-1})					
			Average			Average			Average			
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)				
Substrates												
Ouro Verde	0.256aA	0.268aA	0.286	14..03aB	25.68aA	19.86	50.99aB	101.63aA	76.31			
Antuérpia	0.305aA	0.310aA	0.283	18.24aA	21.41aA	21.33	57.32aB	78.23aA	67.77			
Average	0.281	0.289		16.14	25.05		54.10	89.93				
	Colubrina glandulosa											
	Height (cm)			Stem diameter (mm)			SDB (g^{-1})			RVT (cm^3 cm^3)		
			Average			Average			Average			Average
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)	
Substrates												
Ouro Verde	29.00aB	113.80aA	71.40	9.23aB	25.52bA	17.37	0.89	1.79	1.34	3.24aB	6.50aA	4.87
Antuérpia	34.80aB	187.60aA	111.20	11.55aB	28.54aA	25.05	0.92	1.95	1.43	3.88aB	7.80aA	5.84
Average	31.90	150.70		10.39	32.03		0.91b	1.87a		3.56	7.15	

	RGR (cm cm^2 day^{-1})			TLA (cm^2)			SLA (cm^2 g^{-1})					
			Average			Average			Average			
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)				
Substrates												
Ouro Verde	0.297aA	0.242aA	0.270	41.13aB	83.04bA	62.09	45.25aB	60.99bA	53.12			
Antuérpia	0.276aA	0.229aA	0.252	58.98aB	182.30aA	120.64	67.62aAB	92.32aA	79.97			
Average	0.286	0.236		50.06	132.67		56.43	76.65				
	Plathymenia reticulata											
	Height (cm)			**Stem diameter (mm)**			**SDB (g^{-1})**			**RVT (cm^3 cm^3)**		
			Average			Average			Average			Average
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)	
Substrates												
Ouro Verde	63.00aB	258.80aA	160.90	14.16aB	58.58aA	36.37	1.66aB	18.53aA	10.09	4.81aB	8.73aA	6.77
Antuérpia	53.40aB	234.60aA	144.00	15.14aB	52.33aA	33.74	1.80aB	17.53aA	9.67	4.87aB	9.12aA	6.99
Average	58.20	246.70		14.65	55.45		1.73	18.04		4.84	8.93	
	RGR (cm cm^2 day^{-1})			**TLA (cm^2)**			**SLA (cm^2 g^{-1})**					
			Average			Average			Average			
	6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)		6 month (dry)	12 month (Rainy)				
Substrates												
Ouro Verde	0.282aA	0.360aA	0.321	101.20aB	1303.32aA	702.26	75.20aA	75.03aA	75.11			
Antuérpia	0.276aA	0.305aA	0.290	120.48aB	1160.72aA	640.60	70.90aA	64.87aA	67.89			
Average	0.279	0.332		110.84	1232.02		73.05	69.95				

Values followed by the same lowercase letter in the column and uppercase in the row differ from each other by the student t test 0.05 %.

Table 4. Ratio between variable and maximum fluorescence (Fv/Fm) and Chlorophyll a, b and total of six seedlings of native tree that were cultivated in two types of substrates, one inoculated with AMF and the other not inoculated in the periods of 6 and 12 months (dry and rainy) in the field.

	Pterogyne nitens											
	Fv/Fm			**Chlorophyll *a***			**Chlorophyll *b***			**Total Chlorophyll**		
			Average			**Average**			**Average**			**Average**
	6 month (Dry)	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**	
Substrates												
Ouro Verde	0.668aA	0.746aA	0.707	25.36aA	36.55aA	30.96	12.15aA	12.49aA	12.32	37.51aA	49.04aA	43.27
Antuérpia	0.662aA	0.714aA	0.688	35.35aA	34.83aA	35.09	14.33aA	9.24aA	11.79	49.68aA	44.07aA	46.88
Average	0.665	0.730		30.35	35.69		13.24	10.86		43.59	46.56	
	Schinus terebinthifolius											
			Average			**Average**			**Average**			**Average**
	6 month (Dry)	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**	
Substrates												
Ouro Verde	0.789aA	0.807aA	0.798	37.51aA	39.29aA	38.40	21.04aA	20.06aA	20.55	58.56aA	59.35aA	58.95
Antuérpia	0.792aA	0.803aA	0.798	36.50aA	39.93aA	38.21	18.47aA	21.35aA	19.91	54.97aA	61.27aA	58.12
Average	0.791	0.805		37.01	39.61		19.76	20.71		56.75	60.31	
	Joannesia princeps											
			Average			**Average**			**Average**			**Average**
	6 meses (Dry)	**12 meses (Rainy)**		**6 meses (Dry)**	**12 meses (Rainy)**		**6 meses (Dry)**	**12 meses (Rainy)**		**6 meses (Dry)**	**12 meses (Rainy)**	
Substrates												
Ouro Verde	0.729aA	0.806aA	0.768	34.71aA	34.85aA	34.78	14.03aA	13.45aA	14.34	49.93aA	48.31aA	49.12
Antuérpia	0.766aA	0.775aA	0.771	34.03aA	34.72aA	34.38	15.23aA	13.59aA	13.81	48.06aA	48.31aA	48.19
Average	0.747	0.791		34.37	34.79		14.63	13.52		48.99	48.31	
	Tabermaemontana hystrix											
			Average			**Average**			**Average**			**Average**
	6 month (Dry)	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**	
Substrates												
Ouro Verde	0. 686	0.672	0.679b	34.82aA	34.93aA	34.88	11.09aA	13.26aA	16.67	54.90aA	48.19aA	50.97
Antuérpia	0. 745	0.742	0.743a	35.05aA	37.61aA	36.33	20.08aA	18.18aA	14.64	46.15aA	55.79aA	51.54
Average	0.715	0.707		34.94	36.27		15.59	15.72		50.52	51.99	
	Colubrina glandulosa											
			Average			**Average**			**Average**			**Average**
	6 month	**12 month**		**6 month**	**12 month**		**6 month**	**12 month**		**6 month**	**12 month**	

	(Dry)	(Rainy)		(Dry)	(Rainy)		(Dry)	(Rainy)		(Dry)	(Rainy)	
Substrates												
Ouro Verde	0.750aA	0.797aA	0.774	35.87	32.41	34.14	17.58aA	11.25aA	14.42	53.67	41.26	48.56
Antuérpia	0.752aA	0.797aA	0.774	35.25	30.99	33.12	14.43aA	10.27aA	12.35	49.67	43.67	45.47
Average	0.751	0.797		35.56a	31.70b		16.00	10.76		51.56a	42.46b	
	Plathymenia reticulata											
		Average			**Average**			**Average**				**Average**
	6 month (Dry)	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**		**6 month (Dry)**	**12 month (Rainy)**	
Substrates												
Ouro Verde	0.711	0.758	0.735	32.10	41.69	32.53	18.61aA	23.04aA	20.83	50.71aA	64.73aA	57.72
Antuérpia	0.668	0.771	0.719	29.25	35.80	36.90	20.48aA	19.06aA	19.77	49.74aA	54.86aA	52.30
Average	0.689b	0.764a		30.68b	38.75a		19.55	21.05		50.23	59.80	

Values followed by the same lowercase letter in the column and uppercase in the row differ from each other by the student t test 0.05 %.

Number of glomerospores is modulated according to the periods evaluated

The number of glomerospores in the six native tree species was influenced by the collection period (Figure 2 A-F). For *P. nitens* and *C. glandulosa* in the six-month period, we observed higher sporulation in the Antuérpia substrate compared to Ouro Verde (Figure A and E). In the other plant species, we did not find differences ($p > 0.05$) when unfolding the substrates within each period. When we performed the split as a function of time for each substrate, we found that all native tree seedlings regardless of the substrate had higher sporulation in the six-month period (dry period) compared to 12 months, with the exception of *C. glandulosa* which showed higher sporulation only in the substrate Antuérpia at six months in relation to Ouro Verde and *P. reticulata*, in which Ouro Verde had a higher number of glomerospores compared to 12 months (rainy period).

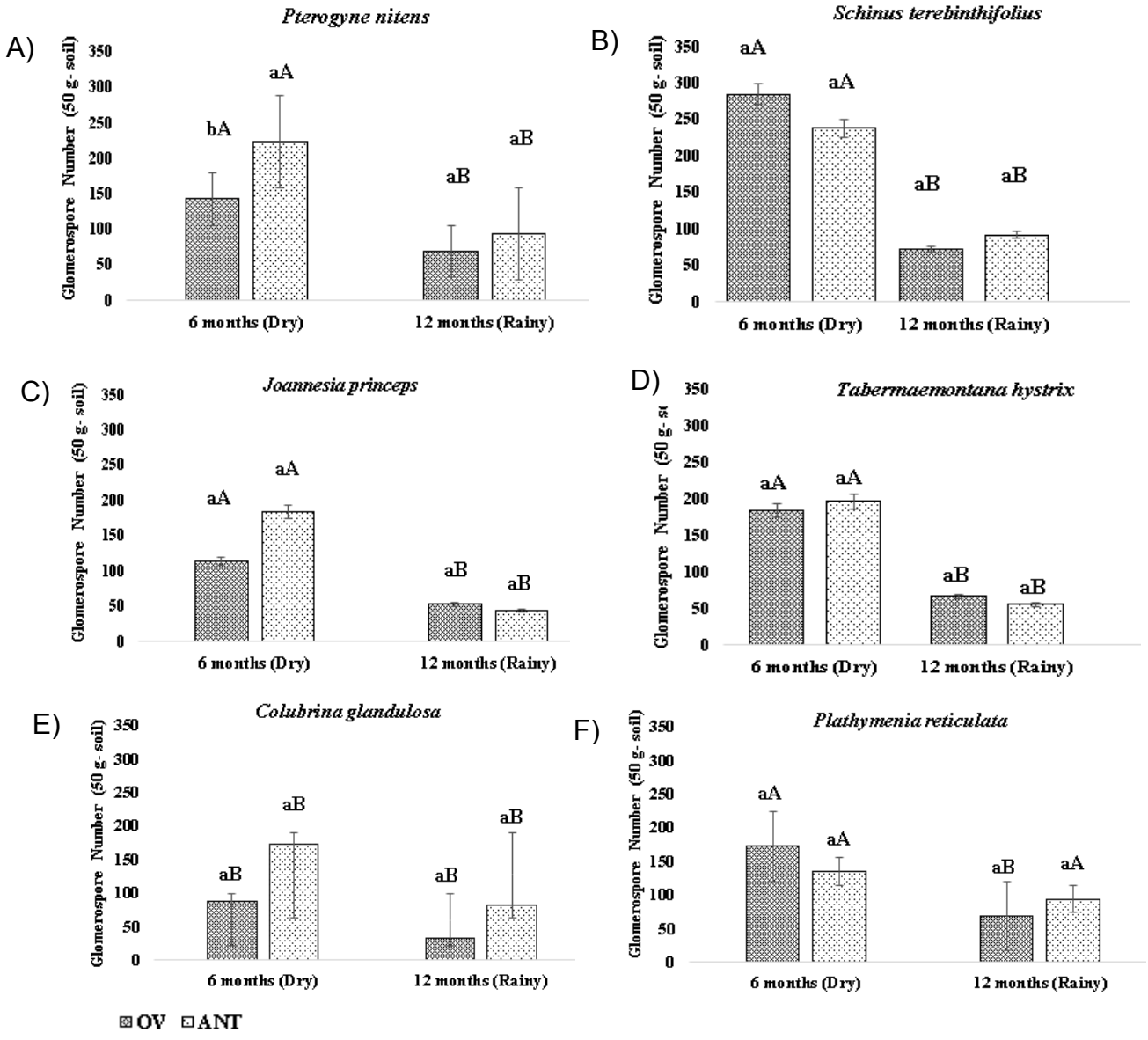

Figure 2. Number of glomerospores in the rhizosphere of six native tree species considering the substrates (Ouro Verde - OV and Antuérpia - ANT) and evaluation periods, 6 months (dry season) and 12 months (rainy season) after the establishment of six native tree species in the field. Values followed by lowercase letters compare the substrates (OV and ANT) within each period and capital letters compare each substrate between the periods (6 and 12 months-dry and Rainy), using the student t test 0.05%.

The period of evaluation influences the chemical and morphophysiological properties of native tree species in the field regardless of the cultivation substrate (multivariate)

The variations in the morphophysiological functioning and in the chemical properties of the soil, after the introduction of seedlings in two types of substrates (inoculated and not inoculated with AMF), were represented in a two-dimensional graph according to principal component analysis (PCA) (Figure 3). In addition, a permutation procedure (PERMANOVA) was performed to correlate the periods (6 and 12 months - dry and rainy). Based on this analysis, it was found that the sampling periods are significantly different regardless of whether the substrate is Antuérpia (inoculated) or Ouro Verde (non-inoculated) (Figure 3).

The first two axes of PCA explained 61.76 % of the variation, in which the first and second variables corresponded to 45.86 % and 15.90 % of the total variation, respectively (Figure 3), with SLA, Fv/Fm, GN, P, K, Ca, Mg, SB, CTC(t) and CTC (T) were positively correlated and Height, SD, Chorl a, b and total, RGR, Al and H+Al, negatively correlated with the first axis, while SD, Chlor a , b and total and RGR are positively correlated with the second axis and SLA, Fv/Fm, pH and H+Al were negatively correlated, suggesting that these variables were influenced by the collection periods (Figure 3; Table 5).

PERMANOVA: ***F: 11.91; P<0.0001***

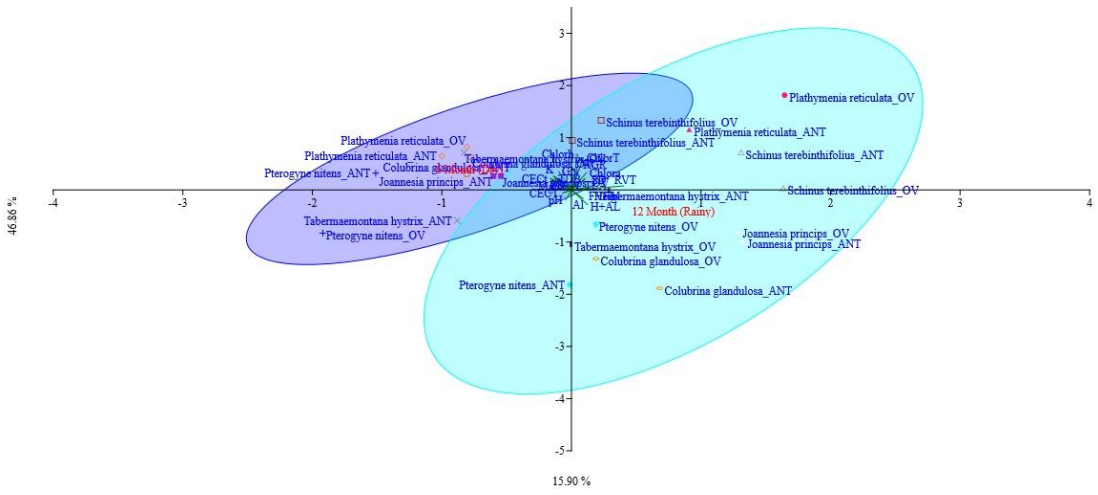

Figure 3. Principal coordinate analysis (PCA) of the relationship between the periods (6 and 12 months - dry and rainy) and the morphophysiological variables and soil chemical properties in six native tree species in the field.

Table 5. Correlation coefficients of the variables analyzed with axes 1 and 2 of the Analysis of Principal Coordinates (CAP) ordination.

Variables	PC1	PC2
Height	-0.899***	-0.008^{ns}
SD	-0.810***	0.368**
SLA	0.409***	-0.156^{ns}
SDB	-0.169^{ns}	0.150 ^{ns}
RVT	0.453***	0.275**
TLA	-0.163 ^{ns}	0.141 ^{ns}
Fv/Fm	0.473***	-0.169^{ns}
Chlor a	-0.684***	0.513***
Chlor b	-0.234**	0.907***
Total Chlor	-0.507***	0.848***
RGR	-0.508***	0.847***
GN	0.540***	0.481***
pH	0.176^{ns}	-0.222**
P	0.822***	0.244**
K	0.846***	0.275**
Ca	0.889***	0.161^{ns}
Mg	0.916***	0.140^{ns}
Al	-0.566***	-0.162^{ns}
H+AL	-0.669***	-0.357**
SB	0.944***	0.195*
CEC(t)	0.942***	0.200*
CEC(T)	0.384**	-0.100^{ns}

ns: not significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.0001$
*SD: Stem diameter; TLA: Total leaf area; SLA: Specific leaf area; SDB: Shoot dry biomass; RGR: Relative growth rate, RVT: Relative volume trunk; GN: Glomerospore Number

Discussion

Changes in soil chemical properties over the evaluation periods were greater at 6 months (dry season) than 12 months (rainy season), which can be due to both low precipitation that led to reduced leaching of soil nutrients, such as cations, soluble compounds ($Ca^{2+,}$ $Mg^{2+,}$ K^{+}), and lower absorption of nutrient in dry season. Furthermore, after 12 months all plants absorve more nutrients, declining soil minerals, coinciding with the period of active development and biomass accumulation by plants (Faturabin and Olojugba, 2014) regardless of the substrate they were produced. These results corroborate a study carried out by Faturabin and Olojugba (2014) which demonstrated that rainfall has a strong effect on the chemical properties of soils in Nigeria. In addition, rainy season can lead to a faster reduction of soil minerals due to high rainfall (Nengi-

Benwari et al., 2021) and there is a positive correlation between rainfall and the growth of tropical tree species (Brienem and Zuidema, 2005).

The beneficial effects of mycorrhizal inoculation were expected, so the Antuérpia substrate promoting better morphophysiological values in all six tree species studied was expected, mainly because they were introduced in a degraded site, as showed by Hart et al. (2018). However, we found that the seasonal conditions of evaluated periods were responsible for the growth changes when considered not only the height, but the volume of trunk, since the shoot architecture differs among plant species. This finding is consistent with the study by Kuster et al. (2017) who observed that the species *Byrsonima verbascifolia* (L.) Rich. presented greater height in the period of greater precipitation. Although *P. nitens* had presented better growth in nursery when produced in Antuérpia substrate, in the field, at 12 months, seedlings produced in Ouro Verde substrate presented better growth, showing that plant species can present different responses. The influence of the type of substrate on the growth of *P. nitens* also corroborates the study by Pacheco et al. (2021).

The leaf functional traits showed distinct responses, varying according to plant species. For *P. nitens* and *J. princeps* species increasing in TLA and SDB was accompanied by a reduction in SLA. At 12 months, these species presented larger leaves and higher biomass, influencing the SLA ($p< 0.05$), producing leaves with smaller area than their leaf mass. Leaves with higher biomass tend to have lower SLA, with a higher cost for light absorption in relation to smaller leaves, as the plant starts to invest more in the development of tissues with greater energy expenditure (Milla and Reich, 2007). High SLA correlates with a higher photosynthetic rate reflecting higher growth and resource capture, however, when the leaf dry mass is higher, the plant starts to grow more slowly (Pérez-Harguindeguy et al. 2013). No increasing in photosynthetic pigments and chlorophyll fluorescence (Fv/Fm) at 6 months was observed. These results were similar to those observed by Gomes et al. (2018) where the Fv/Fm value was up to 0.8 in seedlings of *P.* nitens, recommend its use in programs for the recovery of degraded areas. The higher SLA is interesting after the introduction of seedlings in the field, as it helps in establishment and survival, since SLA tends to be higher in the juvenile stage of plants (Williams-Linera and Marinque-Ascenio, 2020). Additionally, low SLA makes leaves longer and less susceptible to herbivory damage (Lambers et al. 2008; Jager et al. 2015), while increased shoot biomass helps protect against physical damage and predator attacks (Pérez-Harguindeguy et al. 2013).

The inoculated substrate was favorable in promoting the highest SLA in *C. glandulosa* at 12 months (rainy season), while photosynthetic pigments (Chlorophyll *a* and total) showed higher activity at 6 months. A similar response was observed in the nursery, in which *C. glandulosa* seedlings showed better morphological parameters in the Antuérpia substrate (unpublished data, Chapter 1 of the thesis), regardless of inoculation. However, in the field, this substrate, which was inoculated with a mix of AMF species, demonstrated that for this species inoculation was important for increasing SLA, through greater light capture per unit mass. Higher SLA was also observed in *Physalis peruviana* L. seedlings inoculated with a mix of AMF species in saline soil conditions (Miranda et al., 2011). For *C. glandulosa* the greater amount of total chlorophyll *a* at 6 months under drought conditions and greater solar radiation, indicating that under these conditions this plant was able to absorb more light, since chlorophyll is responsible for light capture (Taiz and Zeirger, 2009). Using same plant species, Caus and Paulito (2000) observed that when the environment has low luminosity their photosynthetic efficiency increases because they present a greater amount of chlorophyll *a*. Furthermore, low rainfall does not necessarily affect plant plasticity in a period of low water availability (Kuster et al. 2017).

Fv/Fm values of tree species ranged from 0.6 to 0.8. Although leaves with Fv/Fm values below 0.8 are generally photoinhibited (Luttge et al., 1998), no photoinhibition was observed, may be because of physiological attributes of studied native species. It is value to detached that *S. terebinthifolius* did not present SLA and physiological attributes influenced by season nor substrate. This tree species is considered a plant with high photosynthetic plasticity, so that it can adjust its photosynthetic apparatus to colonize areas with greater environmental instability (Dos Anjos et al. 2015), which is an interesting feature from the point of forest restoration view. According to Franco et al. (2007) some tree species can maintain the functioning of the PSII under conditions of higher irradiance in the dry season. On the other hand, the type of substrate influenced the functioning of PSII (Fv/Fm) of *T. hystrix* seedlings, which differs from the study observed by Afonso et al. (2020), which found no influence of the type of substrate on the physiological parameters of seedlings of *T. catharinensis*. However, the substrate composition is known to influence the physiological apparatus as a function of its chemical constitution and water availability (Gomes et al. 2008). However, P. *reticulata* exhibited a distinct strategy presenting higher chlorophyll *a* content and Fv/Fm in the period of 12 months (rainy season), demonstrating its photosynthetic conditions for capture light due to the large amount of

chlorophyll in its leaves (Shao et al. 2014), although we did not observe differences between the periods regarding SLA.

In all native tree species studied, the highest sporulation occurred in the dry season, corroborating with the literature that the number of glomerospores were higher in the dry season in forested areas (Kamareh et al. 2011; Pereira et al. 2018) compared to the rainy season, which may indicate that during this period of greatest precipitation the glomerospores germinate and thus their number decreases, which suggests that other types of infective propagules (colonized root fragments and external mycelium) can be found in the rainy season (Cuenca et al. 2010). In Cerrado tree species, Vieira Júnior et al. (2020) also observed higher sporulation in the dry season compared to the rainy season and came to the conclusion that mycorrhizal activity is higher during the period of lower rainfall. A study carried out with Moebius-Clune et al. (2013) observes low soil moisture is a determining factor in stimulating mycorrhizal sporulation. Another factor that may have contributed to higher sporulation in the dry season in our study is that during this period we observed greater solar radiation, which consequently may have led to an increase in soil temperature and stimulated sporulation. The effect of sunlight on soil temperature and mycorrhizal sporulation was verified by Menezes et al. (2016), which showed higher sporulation in plants exposed to full sun conditions.

Among six species studied, only *P. nitens* and *C. glandulosa* had mycorrhizal sporulation influenced by the cultivation substrate, with Antuérpia promoting greater sporulation at 6 months. Under nursery conditions, Menezes et al. 2022 (unpublished data, chapter 2 thesis) also observed that the substrate Antuérpia promoted higher sporulation in two native tree species studied in this study. The authors attributed this higher sporulation in this substrate due to the better physicochemical conditions than Ouro Verde. Abaure et al. (2020 and 2021) also observed that the type of cultivation substrate used for seedling production influences mycorrhizal sporulation.

PCA and PERMANOVA reinforced those seasonal variations cause changes in chemical properties (K, Na, Ca, Mg, CEC(t) and CEC(T) and BS), morphophysiological and mycorrhizal in the six native tree species studied. From the multivariate analysis, we found the formation of two groups, in which the height and diameter of the stem were correlated with the period of greater precipitation and the chemical parameters of the soil, the physiological variables and the number of glomerospores were related to the low availability (dry period), reinforcing once again that environmental conditions are the main drivers responsible for the responses and adaptations of plants in the field, regardless of the type of substrate and

inoculation in degraded environments. As observed in other studies (Faturabin and Olojugba, 2014; Nengi-Benwari et al. 2021), soil nutrients can undergo a leaching process as a function of soil moisture in the period of greater precipitation. Regarding plant growth over time, it was expected that at 12 months (rainy season) the plants would show greater development, which corroborates a study carried out by Kuster et al. (2017) with native species of Cerrado. While the physiological attributes showed a greater correlation with the dry season, which differs from most studies observed in the literature in which native tree plants have better physiological conditions in the rainy season (Valladares et al. 2007; Badano et al. 2019).

As for the number of glomerospores, PCA clearly demonstrated that seasonality is an important predictor for mycorrhizal activity, and sporulation was correlated with the dry period. Such results only reinforce what was observed in most field studies with tree species, in which greater mycorrhizal sporulation is observed in the dry season (Guadarrama and Álvarez-Sánchez, 1999; Cuenca et al. 2010; Pereira et al. 2018 and Vieira Júnior et al. 2020) than in the rainy season.

Conclusion

All tree native species studied stablished well in the field. No substrate type used for seedling production affected plant performance in the field. It's worth to remember that although not inoculated, all seedlings were mycorrhized, furthermore the degraded area where seedlings were introduced also present AMF spores. So, it is important to consider mycorrhizal status of seedlings and also AMF in introduction area. Seasonal effect on the growth, physiological and mycorrhizal responses is observed showing higher growth in the rainy season and higher photosynthetic activity in the dry season for some species.

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6. FINAL CONSIDERATIONS

The successful use of symbiotic microorganisms, such as arbuscular mycorrhizal fungi (AMF) in the production process of native tree seedlings is well documented in the literature, mainly by testing specific AMF species, but the influence of the substrate on inoculation efficiency has still been ignored, differing from the conditions adopted in this Thesis. In the study carried out in a nursery, it was noted that the inoculum produced on farm did not present satisfactory results in the initial growth of the 13 native tree species studied, but the inoculation promoted high percentages of mycorrhizal colonization in the roots. In addition, the physical and chemical composition of the substrates during the seedling production stage in the nursery must be considered, as it has a strong influence on plant development, as observed in the present study, in which Antuérpia was the most suitable. Furthermore, the native AMF community is altered as a function of inoculation, and it was possible to understand that some native tree species studied have certain preferences for certain fungal groups.

Finally, in the field, we verified that the use of substrates inoculated with AMF did not influence the growth parameters or the physiological attributes studied, since all seedlings was colonized by AMF and the interaction between biotic and abiotic factors is even more complex under uncontrolled conditions. However, over time, seasonal factors such as time of year strongly alter the physiological responses of plants and mycorrhizal activity in the field.