THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI ON THE EARLY-STAGE RESTORATION OF SEASONALLY DRY TROPICAL FOREST IN CHAMELA, MEXICO

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ABSTRACT – It was evaluated the effect of two different sources of local inocula from two contrasting sites (mature forest, pasture) of arbuscular mycorrhizae fungi (AMF) and a non-mycorrhizal control on the plant growth of six woody species differing in functional characteristics (slow-, intermediate- and fast-growth), when introduced in a seasonally tropical dry forest (STDF) converted into abandoned pasture. Six plots (12 X 12m) were set as AMF inoculum source. Six replicates of six different species arranged in a Latin Square design were set in each plot. Plant height, cover area and the number of leaves produced by individual plant was measured monthly during the first growing season in each treatment. Species differed in their ability to benefit from AMF and the largest responsiveness in plant height and leaf production was exhibited by the slow-growing species Swietenia humilis, Hintonia latiflora and Cordia alliodora. At the end of the growing season (November), the plant height of the fast growing species Tabebuia donnel-smithii, Ceiba pentandra and Guazuma ulmifolia were not influenced by AMF. However, inocula of AMF increased leaf production of all plant species regardless the functional characteristics of the species, suggesting a better exploitation of above-ground space and generating a light limited environment under the canopy, which contributed to pasture suppression. Inoculation of seedlings planted in abandoned pasture areas is recommended for ecological restoration due to the high responsiveness of seedling growth in most of species. Use of forest inoculum with its higher diversity of AMF could accelerate the ecological restoration of the above and below-ground communities.

Keywords: Seasonally tropical dry forest, Arbuscular mycorrhizal fungi and Restoration.

O PAPEL DOS FUNGOS MICCORRÍZICOS ARBUSCULARES NA FASE INICIAL DE RESTAURAÇÃO EM UMA FLORESTA TROPICAL SAZONALMENTE SECA EM CHAMELA, MÉXICO

RESUMO – Avaliou-se o efeito de duas diferentes fontes de inóculo locais (floresta madura e pastagens) de fungos micorrízicos arbusculares (FMA) e de um controle não micorrízico no crescimento de seis espécies arbóreas que diferiam em suas características funcionais (lento, médio e rápido crescimento), introduzidas numa área de floresta tropical sazonalmente seca (FTSS) convertida em pasto abandonado. Foram estabelecidas seis parcelas (12 x 12 m) como duas fonte de inóculo de FMA. Em cada parcela, estabeleceram-se seis repetições de seis diferentes espécies dispostas em delineamento de quadrado latino. Mediram-se mensalmente, durante a primeira temporada de crescimento, a altura, a área de cobertura e o número de folhas produzidas por planta individualmente, em cada tratamento. As espécies diferiram em sua capacidade de beneficiar-se dos FMA, e a maior capacidade de resposta em termos de altura e produção de folhas foi exibida pelas espécies de crescimento lento (Swietenia humilis, Hintonia latiflora e Cordia alliodora). No final do período vegetativo (novembro), a altura de plantas das espécies de crescimento rápido como Tabebuia donnel-smithii, Ceiba

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pentandra e Guazuma ulmifolia não foi influenciada pelos inóculos de FMA. No entanto, a produção de folhas aumentou com os dois tipos de inóculos, independentemente das características funcionais das espécies, sugerindo melhor aproveitamento do espaço acima da superfície e gerando um ambiente limitado de luz sob o dossel, o que contribuiu para a supressão da pastagem. A inoculação das mudas plantadas em áreas de pastagem abandonadas é recomendada para a restauração ecológica devido à sua alta capacidade de resposta do crescimento de plântulas da maioria das espécies. O uso de inóculo de floresta, com a sua maior diversidade de FMA, poderia acelerar a recuperação ecológica das comunidades tanto acima quanto abaixo da superfície.

Palavras-chave: Floresta tropical sazonalmente seca, Fungos micorrízicos arbusculares e Restauração.

1. INTRODUCTION

Among the tropical ecosystems, seasonally dry tropical forest has been recognized as the most endangered and fragile ecosystem (ANZEN, 1988; RINCÓN et al., 1999). At the same time, studies on STDF are underrepresented in the literature compared to studies on tropical wet forests (SANCHÉS-AZOFÉITA et al., 2005). Moreover, works regarding ecological restoration of STDF are even in smaller number than other tropical forests. In 2003, only 3% of restoration studies were carried out in tropical deciduous forests, one of the most abundant vegetation types within STDF (MELI, 2003).

In Mexico, Trejo and Dirzo (2000) estimated that only 27% of the original cover of STDF remained as intact forest, representing about 3.7% of the total area of the country. The main human disturbances in Mexican STDF were associated with conversion to pasture (58%), wood extraction (21%), farming (14%) and forest fires (7%; INE-SEDESOL, 1993). Indeed, STDF regeneration after perturbation is slow and the mortality of seedlings is high (CECCON et al., 2003, 2004), mainly due to the high climatic seasonality (CECCON et al., 2006) and to the severe water limitation resulting from the loss of water retention mechanisms after the removal of forest cover (MAASS, 1995).

Moreover, forest disturbances produced by human activities induce dramatic changes in vegetation and soil (BOLIN; COOK1983; JORDAN, 1986; RAMIREZ-MARCIAL et al., 2001; STERN et al., 2002; CECCON et al., 2002; CECCON; HERNÁNDEZ, 2009). This disturbance is not only above-ground; without natural vegetation, there is also a lack of root activity in the soil and consequently the habitat for arbuscular mycorrhizae fungi is also disturbed. The disturbed areas are generally characterized as sites with low taxonomic diversity of AMF in the soil and low above-ground species diversity (ALLEN, 1991; HELGASON, et al. 1998). Therefore, the early successional species that are naturally pioneer in the regeneration of these habitats generally show a high relative growth rate (Souza; Valio, 2003) and have little or no need for AMF in order to capture nutrients (ALLEN, 1991). Overall, these species either do not form mycorrhizae (non-mycotrophic) or present low responsiveness to them and for this reason may contribute to the strikingly low diversity of AMF in disturbed soils (MILLER, 1979; REEVES et al., 1979; JANOS, 1980). If a non-mycotrophic community or one with low responsiveness is established in an area which has been disturbed, the formation of AMF propagules can be slow, successional processes can be delayed, and the ecological restoration of the disturbed site can be seriously affected.

In contrast, species from late successional habitats generally present a low relative growth rate (GLEESON; TILMAN, 1994) and require the presence of AMF for their development (obligatory mycotrophs; JANOS, 1980). Therefore, plant species within each successional stage differ in their responsiveness to AMF (JANOS, 1980; St. JOHN; COLEMAN, 1983; ALLEN,1991; HUANTE et al., 1993; VAN DER HEIJDEN et al., 1998; KIERS et al., 2000; ALLEN et al., 2003, 2005).

At same time, depending on the successional stage, different AMF species are present (ALLEN, 1991; HELGASON et al., 1998). In a study of the effect of disturbance on diversity of AMF in STDF, Allen et al. (1998) found significantly lower AMF diversity in sites converted to pasture than in mature forest. This may suggest a different functional effect of AMF on plants development depending on AMF species source (successional stage).

The main objectives of the present study were to assess the short-term effects of arbuscular mycorrhizal fungi inoculum from mature forest and pasture on the
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plant growth of six woody species differing in functional characteristics (slow, intermediate, and fast-growing), when introduced in a site converted from seasonally dry tropical forest to pasture. The response variables to be tested were seedling height, number of leaves, and plant cover.

2. METHODS

2.1 Site Description

The experiment was carried at the Chamela-Cuixmala Biosphere Reserve (19°22’ - 19° 39’ N and 104°56’ - 105°10’ W) in a site converted from seasonally dry tropical forest to pasture for cattle grazing 16 years before the experiment. According to Lott et al. (1987) the SDTF in this reserve is characterized by its high plant species diversity (93 species, with 36 tree species with > 10 cm DBH per 1000 m²) and it exhibits a highly seasonal climate with a summer precipitation period from July to October (707 mm annual mean) and average monthly temperature of 24.9°C (mean compiled over 10 years; BULLOCK, 1986; de ITA-MARTINEZ; BARRADAS, 1986). Soils are poorly developed sandy-clay-loams, classified as Eutric Regosols in the FAO system with a pH of 6–6.5 (COTLER et al., 2002). The pasture where the study was carried out is located in a flat area of approximately 8 ha, which is limited on one side by a seasonal river and on the other sides by SDTF.

2.2 Plant species

The criteria to select the woody species used in the experiment considered seedling relative growth rate (RINCÓN; HUANTE, 1994; HUANTE et al., 1995), seed size, and maximum adult height to include a wide range
do functional characteristics (Table 1; LOTT, 1993). Mature seeds of six woody species were collected from at least ten different individuals within the conserved forest, and their average dry biomass (mg) was determined from 50 randomly selected seeds. Seeds were germinated on humid sterile silica sand. Seedlings at five days of age were transplanted in free drainage plastic bags (one seedling per bag) containing 2 kg of a 4:1 mixture of sterilized natural soil and silica sand. The soil was collected from the experimental site and sterilized at 120°C for 48 hours and allowed to rest during four days previous to any plant or AMF inoculation.

2.3 AMF treatments

The two following AMF treatments were applied to seedlings: addition of AMF from the mature forest, and addition of AMF from the pasture. A third treatment (control group), contained no inoculum. In order to obtain AMF spores, soil from the mature forest and from pasture were collected. Soil from each site was mixed with silica sand (1:1) and potted. To promote AMF propagation, grasses were planted in the pots of soil/sand mixture from each site for five months prior to the beginning of the experiment. From these pot cultures, soil containing 1,500 spores and 5 g of grass roots infected with AMF from the forest site or from the pasture site to inoculate the seedlings were used. For the non-mycorrhizal control, sterile soil and roots were added at the same proportion as in the AMF treatments. The inoculated and non-inoculated woody seedlings were grown in a greenhouse for 50 days, with an average peak photosynthetically active radiation (PAR) of 1,500 imol m⁻² s⁻¹ (quantum sensors, LI-190 SB, LI-COR Inc. Lincoln, USA connected to a Campbell 21 XL datalogger) prior to transplant to the experimental field plots.

Table 1 – Characteristics woody species from seasonally dry tropical forest of Chamela, México.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Habit</th>
<th>Seed mass (mg)</th>
<th>RGR g⁻¹ day⁻¹</th>
<th>Maximum adult height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swietenia humilis</td>
<td>Meliaceae</td>
<td>Tree</td>
<td>0.46</td>
<td>0.045</td>
<td>5-20</td>
</tr>
<tr>
<td>Hintonia latiflora</td>
<td>Rubiaceae</td>
<td>Shrub or small tree</td>
<td>1.4</td>
<td>0.075</td>
<td>2-12</td>
</tr>
<tr>
<td>Cordia alliodora</td>
<td>Boraginaceae</td>
<td>Tree</td>
<td>14</td>
<td>0.088</td>
<td>12</td>
</tr>
<tr>
<td>Tabebuia donnell-smithii</td>
<td>Bignoniaceae</td>
<td>Tree</td>
<td>4.7</td>
<td>0.097</td>
<td>4-18</td>
</tr>
<tr>
<td>Ceiba pentandra</td>
<td>Bombacaceae</td>
<td>Tree</td>
<td>116</td>
<td>0.097</td>
<td>25</td>
</tr>
<tr>
<td>Guazuma ulmifolia</td>
<td>Sterculiaceae</td>
<td>Shrub or small tree</td>
<td>4.9</td>
<td>0.126</td>
<td>2-12</td>
</tr>
</tbody>
</table>
Regarding the AMF species found in the pasture and in the mature forest sites, in a previous work in the same study area, Allen et al. (1998) found that spore species composition differed among sites; the predominant AMF in the pasture site was *Glomus magnificaule* which was also present in the forest site but was relatively scarce. The other, a less abundant species found was *Scutellospora pellucida*. On the other hand, in the forest site, 15 AMF species were found in similar abundance: seven *Glomus* species, two *Sclerocystis*, two *Scutellospora* and four *Acaulospora* species.

In the field experiment, on 1-ha fenced pasture, 18 plots of 12 x 12 m (six plots per treatment) were delimited. In order to eliminate grasses, RoundUp® (Ortho®) herbicide was applied to each plot. This herbicide is biodegradable with average lifetime of two to four weeks. There are studies confirming the absence of negative effects of Glyphosate on AMF population dynamics (SOUTH, 1980; JACOBS et al., 2000; MALTY et al., 2006). Transplanting was carried out two months after herbicide application to avoid any toxic effect on plants. Each treatment plot was subdivided to receive 36 seedlings corresponding to six replicates of the six different species (listed in table 1, total of 648 seedlings), which were arranged in a Latin Square design (six rows per six columns). The spacing used among seedlings was 2 x 2 m.

In July (beginning of the rainy period), greenhouse seedlings at 50 days of age were transplanted to field plots to its respective treatment. In order to evaluate plant performance, three different variables of plant growth were evaluated every month from July to November (rainy period). They were the following: i) plant height: from the base of the stem to the base of the apical bud; ii) number of leaves produced, and iii) plant cover: calculated as an ellipse from measurements of maximum and minimum diameter of the crown projection. For all variables evaluated, significant differences among treatments by species were tested separately by repeated ANOVA measurements (p<0.05) and Tukey multiple comparison tests.

### 3. RESULTS

#### 3.1 Plant Height

The six species studied showed different ability to grow in height regardless of treatment (Fig. 1). Regardless of the AMF source, the tallest plants were the fast-growing species *Ceiba pentandra* and *Guazuma ulmifolia*, and the shortest species was the slow-growing species *Swietenia humilis*. Plants grew significantly higher with AMF, regardless of the inoculum source (NI<P=F) for *Swietenia humilis, Cordia alliodora, Hintonia latiflora*, and *Guazuma ulmifolia*, (except July and November in the last two species, respectively). In *Tabebuia donnell-smithii* no significant differences in height among treatments were showed any month (F=P=NI, Fig. 1).

#### 3.2 Leaf production

The production of leaves differed among the species studied. The species are ranked in an increasing order follows: S. *humilis* < T. *donnell-smithii* < C. *pentandra* < H. *latiflora* < C. *alliodora* < G. *ulmifolia* (Fig. 2). By examining the curves generated by plotting the number of leaves produced per time unit, the highest slopes can be found in *Ceiba pentandra* and *Guazuma ulmifolia*, suggesting a higher rate of leaf production than the rest of the species (Fig. 2). After five months of growth, all species presented significant responsiveness to the forest AMF compared with control. In addition, the benefit of pasture inoculum was as strong as that of the forest inoculum in *Tabebuia donnell-smithii* and *Cordia alliodora*. In *Swietenia humilis* and *Guazuma ulmifolia*, the seedlings inoculated with pasture inoculum were not significantly different from the non-inoculated plants. On the other hand, four of the six species studied (*Hintonia latiflora, Cordia alliodora, Tabebuia donnell-smithii* and *Ceiba pentandra*) showed similar leaf production under the forest and pasture inoculum treatments after five months of plant growth.

#### 3.3 Plant Cover

The plant cover described in this section is the result of the amount and rate of leaves produced as well as the amount of branching. *Hintonia latiflora* was the species with the least and *Guazuma ulmifolia* with the most plant cover in all treatments. The maximum plant cover of *Guazuma ulmifolia* was 8000-9500 cm² (November), but no significant differences were found among treatments for this species. Canopy sizes of *Ceiba pentandra* and *Cordia alliodora* were significantly affected by the pasture AMF treatment compared to both forest AMF and control treatments at the end of the rainy season (November). In *Hintonia latiflora*, the forest AMF had a significant effect when compared with other treatments. The other species did not show significant differences among treatments (Figure 3).
Figure 1 – Plant Height (m) attained by the six species during five months under two different sources of AMF inocula, F = forest, P = pasture and a NI= non-mycorrhizal control. Different letters denotes significant differences (p<0.05) among treatments by Tukey test.

Figura 1 – Altura (m) alcançada pelas seis espécies, durante cinco meses, com duas diferentes fontes de inóculo de FMA. F = floresta, P = pastagem e NI = não micorrizadas. Letras diferentes indicam diferenças significativas entre os tratamentos pelo teste de Tukey (p <0,05).
Figure 2 – Number of leaves produced by the six species during five months under two different sources of AMF inocula. F = forest, P = pasture and NI = non mycorrhizal control. Different letters denotes significant differences (p<0.05) among treatments by Tukey test.

Figura 2 – Número de folhas produzidas pelas seis espécies, durante cinco meses com duas diferentes fontes de inóculo de FMA. F = floresta, P = pastagem e NI = não micorrizadas. Letras diferentes indicam diferenças significativas entre os tratamentos pelo teste de Tukey (p < 0,05).
Figure 3 – Plant cover (cm²) attained by the six species during five months under two different sources of AMF inocula. F = forest, P = pasture and NI = non-mycorrhizal control. Different letters denote significant differences (p<0.05) among treatments by Tukey test.

Figura 3 – Área de cobertura alcançada pelas seis espécies, durante cinco meses com duas diferentes fontes de inóculo de FMA. F = floresta, P = pastagem e NI = não micorrizadas. Letras diferentes indicam diferenças significativas entre os tratamentos pelo teste de Tukey (p < 0,05).
4. DISCUSSION

Species differ in their ability to benefit from AMF; previous studies have documented that a large proportion of late-successional tree species presented high responsiveness to AMF (JANOS, 1980; HUANTE et al., 1993, ALLEN et al., 2005) and high levels of AMF colonization whereas the species that typically colonized disturbed habitats presented less responsiveness to AMF (ALLEN et al., 1998; METCALFE et al., 1998, ALLEN et al., 2003). In contrast, in a tropical Brazilian forest, the AMF colonization in the field was 55.5, 26.9, 6.1 and 2.2% for pioneer, early secondary, late secondary, and climax species, respectively (ZANGARO et al., 2000).

In this study, it was found positive responsiveness to AMF inoculation in the plant height of the slowest growth rates species (Swietenia humilis, Hintonia latiflora, and Ceiba alliodora). However, mainly at the end of the growing season (November), the plant height of the fastest-growth rates species (Tabebuia donnell-smithii, Ceiba pentandra and Guazuma ulmifolia) were not influenced by the AMF. On the other hand, AMF inoculation increased leaf production of all plant species and influenced plant architecture of most species regardless of functional characteristics. A higher rate of leaf production has been related to greater light capture, higher CO₂ assimilation (BONGERS; POPMA, 1990) and higher productivity (CHAPIN et al., 2002), which may favor a higher growth rate (POORTER et al., 1990). The previously mentioned results also suggest an influence of the AMF on the ability of the species to explore both vertical and horizontal space. In this sense, a better exploitation of the space as the result of the enhanced growth promoted by AMF inocula may generate a light-limited environment under the canopy and lead to grass suppression in degraded pasture areas. Grass expansion has been known as one of the main biotic barriers to regeneration of native vegetation in pasture areas (HOLL, 1999). Therefore, the inoculation with AMF was beneficial for all species in an abandoned pasture.

Forest disturbance has a negative effect on soil microorganisms, reducing the diversity and composition of AMF (ALLEN et al., 1998; HELGASON et al., 1998; ALLINSON, 2005). Some studies have documented the relevance of the AMF species composition and species richness on plant species composition, variability, productivity and biodiversity, where different AMF species have different responsiveness on plant growth (VAN DER HEIJDEN et al., 1998; KIERS et al., 2000; ALLEN et al., 2003, 2005). In most species evaluated and in most variables measured in this study, the responsiveness to forest AMF inoculum was not significantly different from pasture AMF inoculum, despite the low diversity of the pasture AMF inoculum found by Allen et al. (1998) in our study area. In addition, the overall results highlight the difficulties in generalizing the performance of species established under different AMF inocula - mainly when the objective is seedling production for massive restoration - the use of forest AMF inoculum is recommended, because forest AMF diversity is higher than pasture inoculum (ALLEN et al., 1998) and it could accelerate the ecological restoration of the belowground environment, in addition to improving growth of seedlings.

In practical terms, the production of AMF inocula on a large scale is not possible by using current techniques (FELDMAN; IDCZAK, 1994), therefore establishing a forest AMF treatment, in this case, would be unworkable. However, the reintroduction of small groups of forest AMF-inoculated species in some locations within disturbed areas (called “fertility islands” by ALLEN, 1987) may lead to the establishment of a network of AMF hyphae of high diversity that could accelerate the ecological restoration of the belowground environment, as well as increase seedling growth. An alternative option, for large scale restoration projects, could be the use of a small amount of forest soil in each seedling pot in the greenhouse that may lower the cost of AMF inoculation species and also improve the above and belowground restoration.

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