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Effect of natural nodulation in the development of leguminous trees on soils of cerrado in Tocantins

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ABSTRACT

Experiments were performed in a greenhouse to evaluate the effect of natural nodulation in the development of Pacara Earpod Tree (Enterolobium contorsiliquum) and Leucaena (Leucaena leucocephala) is soil with different uses in Tocantins. We used three soil samples of woods, cultivated areas four and in a degraded area, in pots in a completely randomized design with four replications. In both species nodulation was observed in all soils studies areas with better nodulation occurring in soil cultivation, providing a higher accumulation of biomass. Soil from the degraded area the two species showed nodule number and biomass of nodules significant, with potential for use in disturbed areas, with characteristics of degraded soils.

Key-words: *Enterolobium contorsiliquum*, *Leucaena leucocephala*, native forest species, rhizobium

INTRODUCTION

Environmental degradation in recent decades led to several changes in the environment. The destruction of tropical forests has been happening at an accelerated pace primarily for the sale of hardwood, and replaced by cultivated areas of pasture. Thus, given the magnitude of the action to be taken for restoration of degraded forest ecosystems, particularly along the riparian areas, other areas set a side for permanent preservation (Rodrigues and Gandolfi, 2004) and agroforestry systems, selection of species has a more rustic great importance not only to ensure survival in the field, but also to provide the proper environment for the emergence of other species in order to facilitate plant succession and reverse the degradation process (Soares and Rodrigues, 2008). Reforestation practices and restoration of soils with leguminous trees constitute option in the recovery of degraded area sand the establishment of ecological corridors in the Cerrados of Southern Tocantins. Many of these trees have rapid growth, multiple use, are easy to spread, have the potential to genetic increase and ecological significance for biological nitrogen fixation (FBN) (Franco and Faria, 1997). The use of introduced legume trees will form nodules and will benefit from BNF, in a given location, if native populations of rhizobia in the soil are compatible. The efficiency of the process, however, depends on factors of the plant, bacteria, climate and soil. Leguminous trees have specific result of the recognition process between the plant and bacteria. Through the selection of efficient strains of rhizobia is possible to produce seedlings of forest and nodulated, with faster growth and resistance to field conditions. Encouraging the planting of leguminous trees, as alternatives to reclaim land for reforestation and must be preceded by research to assess the potential for nodulation of the species in local conditions and the consequent prospect of soil microorganisms with potential for use as inoculants. According to Souza et al. (2007) to explore for natural nodulation of tree species in local soils, associated with a program of exploration of new strains, with subsequent application of tests with strains agronomic

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potential and recommended strains are important for definition of which species can be planted for the recovery of disturbed areas. Thus, because of differences in the ability nodulation of leguminous trees with native strains, bioprospecting of rhizobia in soils it escan provide information about the efficiency of natural nodulation and nitrogen fixation of the species. In addition to providing the economy with nitrogen fertilizer, an important aspect for less capitalized farmers in tropical regions (Roy et al., 2002), the introduction of native plant communities managed in association with micro-symbionts, such as rhizobia, is a promising biotechnological tool to assist in there population of degraded ecosystems (Requena et al., 2001), as well as the first step for studies on the ecology of microorganisms (Silva et al.,2011), that is, where they occur and the frequency of their occurrence. The aim of this study was to evaluate the natural nodulation and seedling growth of Pacara Earpod Tree (*Enterolobium contorsiliquum*) and leucaena (*Leucaena leucocefala*) in soils with different forms of use in the Cerrado of Southern Tocantins.

MATERIAL AND METHODS

We collected eight soil samples (0-20cm) in the counties of Gurupi, Nativity and Pedro Afonso in Tocantins, between September and October 2010, featuring covers of the soils, and obtained four soil samples from the agricultural area, three samples forest soil and as oil sample of degraded areas (Table 1). The samples were air dried, bolted in mesh of 2 cm and distributed in plastic bags measuring 3 kg. Were determined the chemical properties of soils used (Embrapa, 1997) (Table 2), Laboratory of Soil in the UFT Campus Gurupi. The pH was determined in water (1:2.5); Ca, Mg and Al were extracted with KCl 1N; K was determined by flame photometry; the P was extracted with the Mehlich 1 and measured by spectrophotometer. To determine the presence of rhizobia that may nodular leguminous trees, in soil samples, two in dependent experiments were conducted in a greenhouse at the Federal University of Tocantins, Campus of Gurupi, November 2010 to February 2011, with the following species of tropical leguminous (*Leucaena leucocephala* (Lam.) De Wit. Mimosoideae, Eumemoseae) and Pacara Earpod Tree (*Enterolobium contorsiliquum* (Vell.) Morong). Seeds were collected from matrixes from the region. The seeds of leucaena were submitted to thermal shock, with boiling water followed by immersion in water at room temperature for 24 hours. The seeds of pacara earpod tree were scarified with sand paper up to visible wear of their teguments then they were immersed in water for 24 hours. Then they were planted 1 cm deep intrays with sand was hed and autoclaved. At 15 days after sowing, two Leucaena seedlings were transplanted and a pacara earpod tree to the polyethylene bags with a capacity of 10 kg of substrate. We made measurements of stem diameter and stem length, using a digital caliper and a ruler, being made at 20, 45, 75 and 105 days after transplanting. Irrigation was effect eddaily with the use of micro sprinklers until it reached, approximately 80% of field capacity, besides the removal of invasive plants. After harvest at 105 days, we determined the dry weight of shoot, root and total number of nodules and dry weight after drying in an oven at 65 °C for 72 hours. To preserve the original condition of the soils used in the experiments, fertilization was not made. The experimental design was completely randomized with eight treatments (soil) and four repetitions, being made in the Tukey comparison of meansuring the program Assistat.

Table 1. Location and description of forms of land use in the study areas in the cerrado of the State of Tocantins.

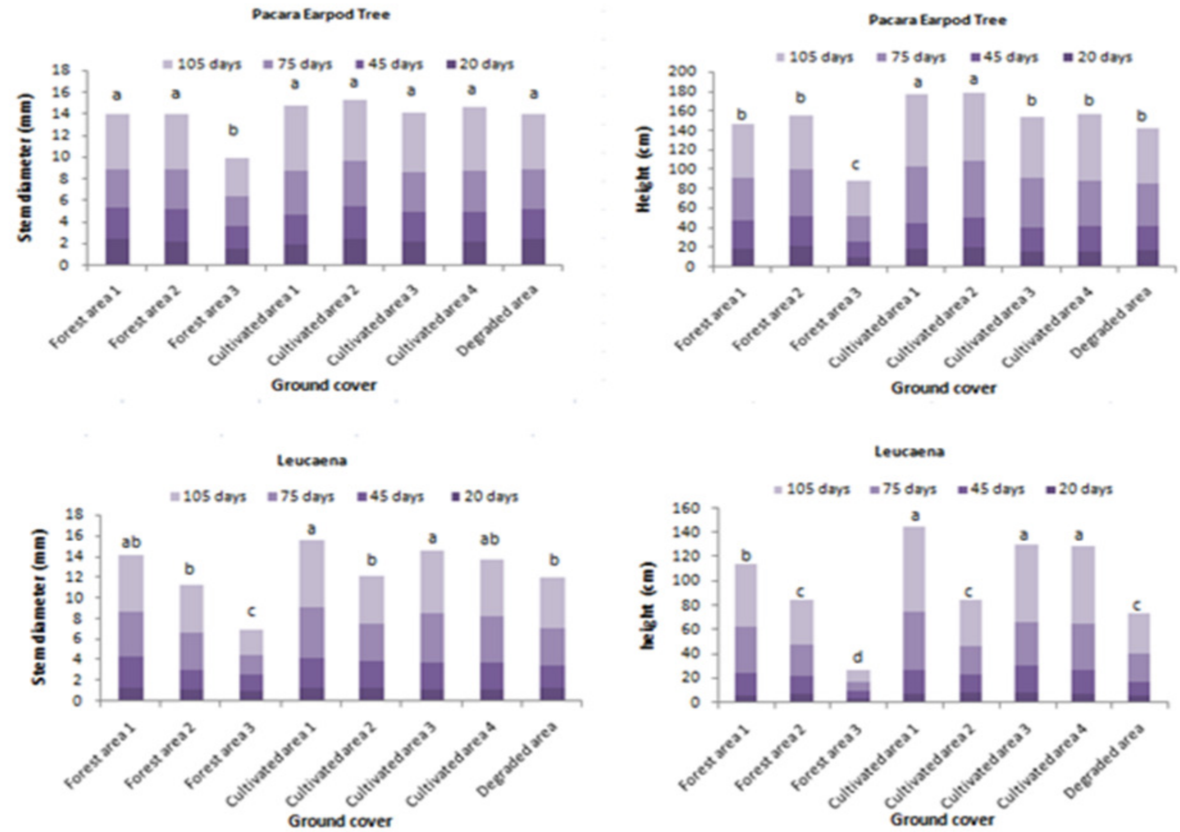
Area/ Town	Geographic coordinates	Description of the sampled area
Forest area		
1. Gurupi	11º43´45´´S 49º04´07´´W	Experimental station of the UFT in Gurupi. Trees over 6 feet tall, within the UFT.
2. Natividade	11º42´35´´S 47º43´24´´W	Area of secondary forest, Agrothechnical School Nativity, with woody vegetation in the process of plant succession.
3. Pedro Afonso	08º58´03´´S 48º10´29´´W	Area of secondary forest in Agrotechnical School Nativity, with woody vegetation in the process of plant succession.
Cultivated area		
1. Gurupi	11º43´45´´S 49º04´07´´W	Experimental station of the UFT in Gurupi. Areas with a history of planting corn, soybeans, sugarcane and cowpea, with presence of invasive plants.
2. Natividade	11º42´35´´S 47º43´24´´W	Growing area Agrothechnical School Nativity, by planting cowpea, maize and velvet bean, without the presence of invasive plants.
3. Pedro Afonso	08º58´03´´S 48º10´29´´W	Area by planting crotalaria as green manure, with the presence of invasive plants.
4. Gurupi	48º59´00´´N 48º58´00´´W	Growing area in the Green Valley Settlement 20 km from Gurupi, with cultivation of maize and cowpea with the presence of invasive plants.
Degraded area		
1. Gurupi	11º43´45´´S 49º04´07´´W	Experimental station of the UFT in Gurupi. Area with a history of degraded pasture.

Table 2. Chemical characteristics of soils collected in areas with different forms of land use in different counties in the Cerrado of Tocantins.

Ground Cover	pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}
	H_2O	mg/dm^3	-----------$cmol_c/dm^3$------------			
Forest area1 (Gurupi - UFT)	5,3	3,3	0,1	6,2	0,1	0,1
Forest area2 (Natividade)	5,5	3,1	0,1	1,2	0,15	0,2
Forest area3 (Pedro Afonso)	5,1	3,2	0,1	1,2	0,1	1,2
Cultivated area1 (Gurupi - UFT)	5,6	13,7	0,15	3,5	1,29	0,18
Cultivated area2 (Natividade)	5,4	3,1	0,1	1,7	0,1	0,2
Cultivated area3 (Pedro Afonso)	5,1	3,3	0,1	0,3	0,1	0,5
Cultivated area 4 (Gurupi - rural settlement)	5,6	6,7	0,1	2,4	0,88	0,2
Degraded area (Gurupi - UFT)	4,6	2,2	0,1	0,8	0,1	0,4

RESULTS AND DISCUSSION

The diameter of the seedlings of pacara earpod tree was favored in soils of forest areas, cultivated and degraded areas, ranging from 5.01 to 5.97mm, with the exception of forest soil three which was significant lower (p<0.001), with 3.57mm. About the seedlings of Leucaena was observed higher values for stem diameter for soils of forest area 1 and cultivated areas 1, 3 and 4, with 5.49, 6.41, 6.0 and 5.49mm, respectively, superior (p>0.001) the other soils studied. The growth in stem length (height) for the species pacara earpod tree was favored in soils with planted areas, compared to other soils (Figure1). In these soils the seedlings pacara earpod tree reached between 62.5 and 74.3 cm. For leucaena values were higher (p<0.001) for treatments with soil from cultivated areas 1, 3 and 4 (Figure1).



For the shoot biomass was observed little plant growth in soils of pacara earpod tree sampled in forest areas and degraded, with higher values for the growing areas (p>0.001). For the species *L. leucocephala*, similar results were observed for shoot biomass and total, especially for a growing area. For both species were observed lower values of biomass from the total area and with soil of the forest area 3 (Table3). For the pacara earpod tree species, t was found a smaller root development in forest soil 3, while for all leucaena area of forest land, degraded area and cultivated area 2 were significantly lower than other areas of cultivation. Similar results were reported by Souza et al., (2007) found that less root development of leucaena, which shows greater difficulty in establishing this species in soils of forest areas. The development of roots of leucaena is sensitive to soil acidity and the aluminum content, as noted by Vale et al., (1996) and Silva et al., (2009), which may explain the poor development of roots, a characteristic found for samples of forest soil used in the experiment. In both species were observed nodulation in all soils of the areas studied, with the best nodule formation in these species occurring in soil cultivation 1 and 4. It was found low nodulation and dry biomass of the nodules, also, for solo of the forest area 3, which may have reflected in downtown biomass accumulation of Part area and total (Table 3). This low nodulation in soils without addition of rhizobia, is explained by the high host specificity of leucaena. Species specificity in relation to rhizobia are more responses to inoculation with selected strains that promiscuous species. Species such as leucaena can nodular with more than one kind of rhizobia, and in Brazil are suitable strains of *Rhizobium* and *Bradyrhizobium elkanii* sp. for use as inoculants for this species (Memma et al., 2006).

In these species, the above ground biomass in a forest soil exceeded the remaining areas of forest. The soils of the area of cultivation, especially the cultivation of soils 1 and 4, which showed the potential for adaptation to this soil class. The pacara earpod tree species had the highest average growth shoots and roots in relation to the species *L. leucocephala*. It was observed that the cultivation areas 1 presented greater number of nodules for the two species under study, followed by cultivation area 4, when compared with the other areas (Table1). This maybe due to higher pH and lower presence of Al^{3+} concentrations (Table 3). The soil cultivation 1 and 4 showed higher pH, higher content of and less Al^{3+}. When considering the variables of growth, biomass and nodulation analyzed for Pacara Earpod Tree and leucaena, it is possible to classify the ecological potential of species for N2 fixation and efficiency of natural nodulation in soils in the Cerrado of southern Tocantins. However, the potential for nodulation by these species has not been fully displayed, possibly due to unfavorable environmental conditions such as pH, Al^{3+} and phosphorus levels in soil (Zahran, 1999, Figueiredo et al., 2008), considered abiotic factors affecting survival and nodulation by rhizobia.

Table 3. Dry matter weight of the aerial parts (DMWAP), root dry weight (RDW), total dry weight (TDW), nodule number (NN) and nodule dry weight (NDW) of Pacara Earpod Tree and leucaena seedlings in soil collected from areas with different forms of use land, the Cerrado of Southern Tocantins, to 105 days after transplanting.

Ground Cover	DMWAP ($g\ vase^{-1}$)	RDW ($g\ vase^{-1}$)	TDW ($g\ vase^{-1}$)	NN	NDW ($g\ vase^{-1}$)
Pacara Earpod Tree					
Forest area 1	7,2 bc	8,9 ab	16,2 bc	30,8 ab	41 ab
Forest area 2	6,3 cd	7,6 ab	13,9 cd	23,8 abc	30,5 ab
Forest area 3	3,5 d	4,2 b	7,7 d	8 c	8,3 b
Cultivated area 1	12, 6 a	11,3 a	23,9 a	42,3 a	56,3 a
Cultivated area 2	10,6 ab	10,6 a	21,2 abc	22 abc	29,5 ab
Cultivated area 3	9,5 abc	12,3 a	21,8 ab	19 bc	24,5 ab
Cultivated area 4	10,7 a	10,5 a	21,2 abc	41,8 a	49 a
Degraded area	6,8 cd	12,3 a	20,1 abc	23 abc	37,5 ab
F Test	16,15**	7,17**	10,69**	6,88**	4,51**
CV (%)	17,39	21,15	17,87	33,52	40,73
Leucaena					
Forest area 1	9,05 c	4,36 b	13,40 c	26,5 abc	33,8 ab
Forest area 2	6,29 c	4,48 b	10,77 c	16,5 bc	39,8 ab
Forest area 3	1,99 d	1,49 b	3,55 d	8,0 c	10,3 b
Cultivated area 1	21,35 a	11,74 a	33,11 a	41,3 ab	50,5 a
Cultivated area 2	7,54 c	4,34 b	11,88 c	22,5 abc	29,0 ab
Cultivated area 3	15,01 b	10,44 a	25,42 b	38,3 ab	37,8 ab
Cultivated area 4	16,69 b	9,51 a	26,22 b	49,5 a	54,3 a
Degraded area	5,95 c	4,41 b	10,36 c	25,8 abc	40,8 ab
F Test	79,72**	18,66**	73,78**	5,49**	3,17*
CV (%)	13,95	26,82	14,01	40,93	41,19

Means followed by the same letter in columns are not significant by Tukey´s test ($p<0.001$).

Studies with the use of leguminous species for revegetation of disturbed areas with degraded soils in southern Tocantins are scarce. Tree legumes can prevent erosion, improve soil fertility and facilitate the establishment and growth of other plant species (Cross and Schlesinger, 1999; Rodríguez-Echeverría and Pérez-Fernández, 2003). Furthermore, inoculation of seed or seedlings with rhizobia strains natives appropriate can guarantee the nodulation of roots, increase performance of plants and reintroduce these microorganisms in soil (Requena et al., 2001; Rodríguez-Echeverría and Pérez-Fernández, 2005). Thus, the information obtained in this study help in the screening of species for different purposes, such as species of high ability for nodulation and nitrogen fixation, as a priority in reforestation. However, for better implementation of the leguminous in forest restoration, the main factors affecting the performance of nodulation and growth potential of these species should be evaluated in field conditions. Thus, there is a need to conduct experiments inoculated rhizobia strains isolated for these species in greenhouse and field conditions in terms of climate and soil of the Cerrado in Tocantins.

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CONCLUSIONS
The growth, development and nodulation of the studied species were favored in the soil of the agricultural area. The Pacara Earpod Tree was more efficient nodulation with native populations of rhizobia.

RESUMO
Foram realizados experimentos em casa de vegetação para avaliar o efeito da nodulação natural no desenvolvimento de tamboril (*Enterolobium contorsiliquum* (Vell) Morang. e Leucena (*Leucaena Leucocephala*) em solos com diferentes usos no Tocantins. Foram utilizadas três amostras de solos de mata, quatro de áreas cultivadas e uma de área degradada, em vaso em delineamento inteiramente casualizado com quatro repetições. Nas duas espécies estudadas foi observada nodulação em todos os solos das áreas estudadas, com melhor nodulação ocorrendo nos solos de cultivo, proporcionando maior acumulo de biomassa vegetal. No solo de área degradada as duas espécies apresentaram número de nódulos e biomassa dos nódulos significativos, apresentando potencial para utilização em áreas alteradas, com características de solos degradados.
Palavras-chave: Enterolobium contorsiliquum, Leucaena leucocephala, espécies nativas florestais, rizóbio

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