

Development and Leaf Morphofunctional Attributes of Native Species Used in Oil Well Base Revegetation

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Abstract

This study aimed to evaluate the influence of soil preparation and mineral fertilization on according to the morphological growth and leaf morphofunctional attributes of native species used in the revegetation of the well base area of oil extraction. The experimental design was in randomized blocks in a split-plot scheme with 4 replications. The plots were the two soil types, and the subplots were the 5 planting fertilization treatments with NPK 04-14-08 with 0, 40, 80, 160 and 320 g pit⁻¹. Four native species were planted and individually evaluated. The species responded in a variable way depending on the applied fertilization. The type of soil statistically influenced the number of leaves of *Inga laurina*, showing a greater number when cultivated in clayey soil. The other species did not differ in terms of soil type. For planting fertilization, it is recommended to apply 219.27 to 227.25 g pit⁻¹ for *Schinus terebinthifolius* Raddi and 189.83 g pit⁻¹ for *Mouriri guianensis*. The application of planting fertilizer for *S. terebinthifolius* Raddi and *M. guianensis* is recommended. The species *Inga laurina*, *Garcinia brasiliensis* and *Chrysobalanus icaco* developed better without planting fertilization. Leaf attributes demonstrated an adaptive response of plants regarding to environmental stress conditions to which they were submitted.

Keywords: degraded area, fertilization, rehabilitation, restinga, leaf attributes, adaptive response

1. Introduction

Oil production in the continental onshore area is a significant economic activity in some Brazilian states (Martins et al., 2015). However, like all extractive activities, besides the benefits, it also impacts on the environment negatively. Deforestation, fragmentation of ecosystems, relief alteration and erosion processes are some of these impacts (Martins et al., 2015; Kanashiro & Miranda, 2016).

In Brazil, several oil wells are found in coastal areas, belonging to the Atlantic Forest biome, which occupies about 15% of the total Brazilian territory. With the processes of colonization and exploration, this biome now has approximately 12.4% of its original composition (INPE, 2020). Among the several ecosystems that compose the Atlantic Forest, we find the Restinga, which is a large phytogeographic complex composed of a plant formations mosaic (Silva & Melo Júnior, 2017).

Restinga vegetation is severely damaged by increasing urbanization, tourism, agriculture, extractive activities and deforestation (Freitas et al., 2019). Oil exploration and production in Espírito Santo dates back to the 1960s, when environmental licensing still did not consider restinga as a Permanent Preservation Area (PPA) (Conama, 2006).

Therefore, several activities of exploration and production of oil were installed in these areas, according to the applicable legislation. In these environments, vegetation and surface layers of fertile soil were removed for the construction of well bases, oil treatment units and side roads. These installations required soil compaction with the proper use of clay to cover the areas, which negatively impacted soil microbial life. However, the deposited soil (clayey) and the restinga natural soil (sandy) are very different. Clay soils are characterized by having a greater amount of organic matter and greater water storage capacity, besides having greater variability in fertility, compared to sandy soils. Moreover, clay soils are more susceptible to compaction, which can hinder the penetration of plant roots (Centeno et al., 2017).

Thus, to mitigate the negative impacts caused in this environment, recovery work has been developed in impacted areas through revegetation. The criteria for planting seedlings consider the use of pioneer and non-pioneer species (Boaventura et al., 2019). Impacted soils require a source of nutrients to boost the growth of species, especially in the initial phase (Moreira et al., 2019). Accordingly, this study aimed to evaluate the influence of soil and mineral fertilization, according to the growth and leaf morphofunctional attributes of native species used in the revegetation of the base area of a deactivated oil extraction well.

2. Methodology

2.1 Study Site

The experiment was developed from January 2019 to January 2020, in a coastal plain area. The native vegetation of the region is characterized as restinga vegetation, with a predominance of sandy soil. The experimental area was installed on a deactivated oil well base. During the implantation of these wells, a 20 cm-layer of clay soil from loan areas in the region was added to favor the aggregation of the soil and provide support for the passage of vehicles and the installation of the necessary equipment for the extraction and production of oil. Due to this process, the deposited soil was compacted during the construction of the base, and the original soil of the area was affected with the removal of the entire fertile layer.

2.2 Experimental Design

The experimental design used was in randomized blocks in a split plot scheme with four replications. The plots constituted of two soil types (sandy and clayey) and the subplots by the five planting fertilization treatments with NPK 04-14-08 (0, 40, 80, 160 and 320 g pit⁻¹). Each experimental plot measured 100 m² (10 × 10 m). Each experimental unit was represented by three plants.

2.3 Plant species

The species selected for planting were: *Schinus terebinthifolius* Raddi (Aroeira), *Chrysobalanus icaco* L. (Guajiru), *Inga laurina* (Ingá Mirin), *Mouriri guianensis* (Murta) and *Garcinia brasiliensis* (Bacupari). The native seedlings were purchased at the José Bahia Environmental Socio-Cultural Center, São Mateus, ES, Brazil.

2.4 Soil Preparation

Soil preparation was conducted throughout the experimental area. For the clay plots, subsoiling and harrowing was performed to unpack the soil. In the plots consist of sandy soil, all residual clay surface layer was removed, using a tire tractor and backhoe. Then, Glyphosate® herbicide (1.5 L ha⁻¹) was applied (15 days before planting) in all experimental plots to eliminate weeds. Subsequently, the pits were prepared for planting, approximately 20 cm depth, fertilized with NPK (04-14-08) according to the treatments.

2.5 Planting and Cultural Treatments

One hundred and twenty seedlings of each native species were planted with a spacing of 1.33 × 1.00 m. To protect against high temperatures, the seedlings were covered with coconut straw for 3 months after planting, and those that died up to one month after planting were replanted. These losses were not accounted in the survival rate, since it is an activity normally carried out in revegetation programs.

During the first 3 months, drip irrigation was conducted with 45-minute watering shifts, twice a day, with a total supply of 3.0 L of water per day per plant. At 3 months, to provide greater adaptation of plants to environmental conditions, irrigation was rescheduled to 3 times a week. Total irrigation shutdown occurred 5 months after transplanting.

All plants received cover fertilization with 25 g pit⁻¹ of NPK 20-00-20 with micronutrients, being in the first year every 60 days from planting. Whenever necessary, weed control was conducted throughout the experimental area, by plant weeding and crowning. The control of leaf-cutting ants was done using Mirex-SD® ant killer baits, spread close to the anthills.

2.6 Ecophysiological Assessments

2.6.1 Survival and Growth

Growth assessments were conducted at six months after planting. The evaluated variables were: plant height (PH), measured with the support of a measuring tape graduated in millimeters; stem diameter (SD), measured at ground level by a digital pachymeter and leaf number (LN), obtained by counting all developed leaves. The survival rate, expressed as a percentage, was assessed at three months and one year after planting.

2.6.2 Foliar Attributes

Leaf attributes were evaluated six months after planting, with collection of two leaves per treatment in each block, stored in paper bags and placed in a polystyrene box with ice, to avoid excessive water loss. Then, in the laboratory, a metal perforator was used to remove three discs of 27.99 mm² in diameter from each of the median region of the leaf blade.

With the aid of a precision digital scale (0.0001 g), the leaf discs were weighed to obtain the fresh mass value (FM). Then, the discs were placed in Petri dishes and hydrated with distilled water for 24 hours. Subsequently, they were weighed to obtain the turgid mass value (TM) and measured with a digital pachymeter (Digimess® 100.174BL 150mm/6) to obtain the leaf thickness (LT). Finally, the discs were packed in paper bags and placed in an oven for drying at 60 °C until constant weight, obtaining the dry mass (DM). From these values, the following characteristics were estimated: succulence (g m⁻²) (SUC), leaf mass by leaf area (g m⁻²) (LMA) (Kluge and Ting, 1978), and sclerophilia index (g mm²) (Rizzini, 1997), in which:

$$\text{Succulence} = TM - DM / \text{Leaf area} \quad (1)$$

$$\text{Leaf mass per leaf area} = DM / \text{Leaf area} \quad (2)$$

$$\text{Sclerophilia index} = (DM/2) \cdot (\text{Leaf area}) \quad (3)$$

2.7 Statistical Analysis

The data were subjected to analysis of variance and, when significant, the Tukey mean test at 5% was used for qualitative treatments (soil types) and linear and quadratic regression for quantitative treatments (fertilization doses). The statistical analysis was performed using the Sisvar® Software (Ferreira, 2011).

3. Results

3.1 Survival Rate

The plant survival rate was 100% for all species at 3 months after planting. After one year, variations were observed according to the species and treatments applied (Table 1). *S. terebinthifolius* Raddi maintained a 100% survival rate for all treatments, whereas *C. icaco* had only 58.33% of the plants in the sandy soil at a dose of 320 g pit⁻¹ of NPK. However, this value reduces when the plants were submitted to clayey soil, with only 8.33% of survival. Concerning the *I. laurina*, 100% survival was observed in most treatments, except in the sandy soil/320 g, clayey/40 g and clayey/160 g treatments, with 91.67% survival. *M. guianensis* species showed a high rate of survival in the field after one year of planting, with most treatments maintaining 100% survival, with the exception of plants grown in clayey soil/160 g pit⁻¹ of NPK, for which 83.33% survival was recorded. The survival index obtained for the *G. brasiliensis* species in the field was average in the clayey/160 g treatment, presenting 50% survival, followed by 75% in the clayey/320 g treatment, 83.33% in sandy/320 g, clayey/40 g and clay/80 g and high in the other treatments, ranging from 91.67% to 100% for the sandy treatment/160 g.

Table 1. Survival index of *Schinus terebinthifolius* Raddi (St), *Chrysobalanus icaco* L. (Ci), *Inga laurina* (Il), *Mouriri guianensis* (Mg) and *Garcinia brasiliensis* (Gb), three months and one year after planting in oil well base revegetation

Treatment/g NPK 04-14-08	3 months	1 year				
	All species	St	Ci	Il	Mg	Gb
Sandy/0	100.00	100.00	100.00	100.00	100.00	91.67
Sandy/40	100.00	100.00	100.00	100.00	100.00	91.67
Sandy/80	100.00	100.00	100.00	100.00	100.00	91.67
Sandy/160	100.00	100.00	75.00	100.00	100.00	100.00
Sandy/360	100.00	100.00	58.33	91.61	100.00	83.33
Clayish/0	100.00	100.00	91.67	100.00	100.00	91.67
Clayish/40	100.00	100.00	91.67	91.67	100.00	83.33
Clayish/80	100.00	100.00	91.67	100.00	100.00	83.33
Clayish/160	100.00	100.00	75.00	91.67	83.33	50.00
Clayish/320	100.00	100.00	8.33	100.00	100.00	75.00

3.2 Plant Growth

3.2.1 *Schinus terebinthifolius* Raddi

By analyzing the two factors in isolation, was observed that the soil factor did not differ for any of the variables analyzed. However, the fertilization factor showed a significant difference at six months for the variables plant height, stem diameter and leaves number. The analysis showed no significant interaction between the soil and fertilization factors for the evaluated growth variables. Regarding the quantitative factor (fertilization), NPK doses applied at planting influenced the three growth variables evaluated (PH, SD and LN). The behavior of PH was best explained by the increasing linear regression model, with a determination coefficient of 0.6325. Plant height (cm) increased gradually according to the supply of NPK doses at planting (Figure 1A). The data for the SD and LN variables were better adjusted to the quadratic model (Figures 1B-1C). Regarding the DC variable, a determination coefficient of 0.8191 was observed, with a maximum point of 17.14 mm reached at a dose of 210.5 g pit⁻¹ (Figure 1B). The largest leaf number was obtained at a dose of 227.25 g pit⁻¹ of NPK, with an average of 177.22 leaves per plant and determination coefficient equal to 0.6709 (Figure 1C).

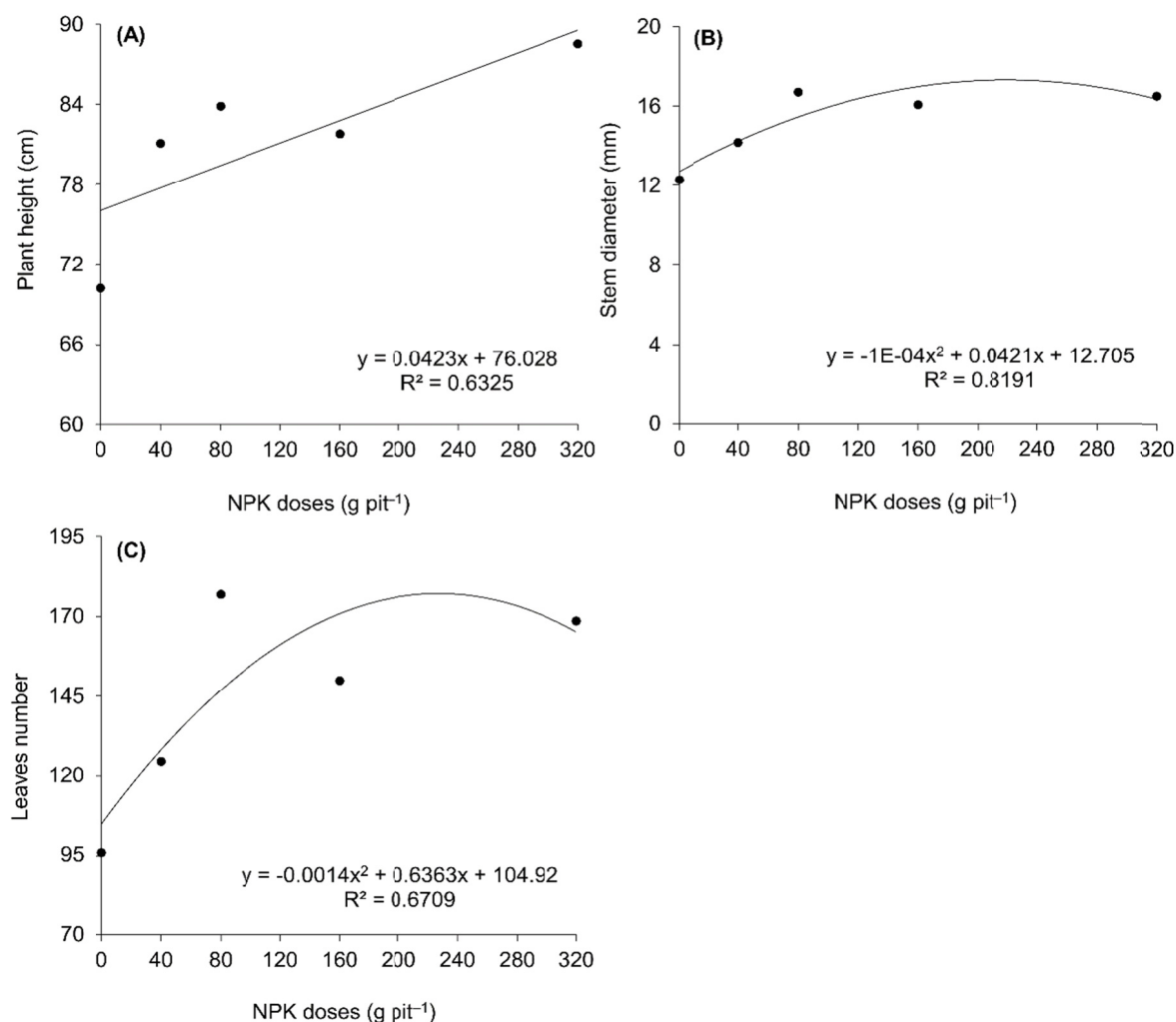


Figure 1. Effect of planting fertilization with NPK 04-14-08 on plant height (A), stem diameter (B) and leaves number (C) of *Schinus terebinthifolius* Raddi, six months after planting in an oil well base area

3.2.2 *Chrysobalanus icaco*

The sandy and clayey soils showed no difference for the growth characteristics of the plants; however, the fertilization treatment showed a significant difference between the doses tested for all variables (PH, SD and LN). The regression model that best fitted the data for the three growth variables was the decreasing linear regression (Figure 2). The treatments tested showed no interaction for any of the growth variables.

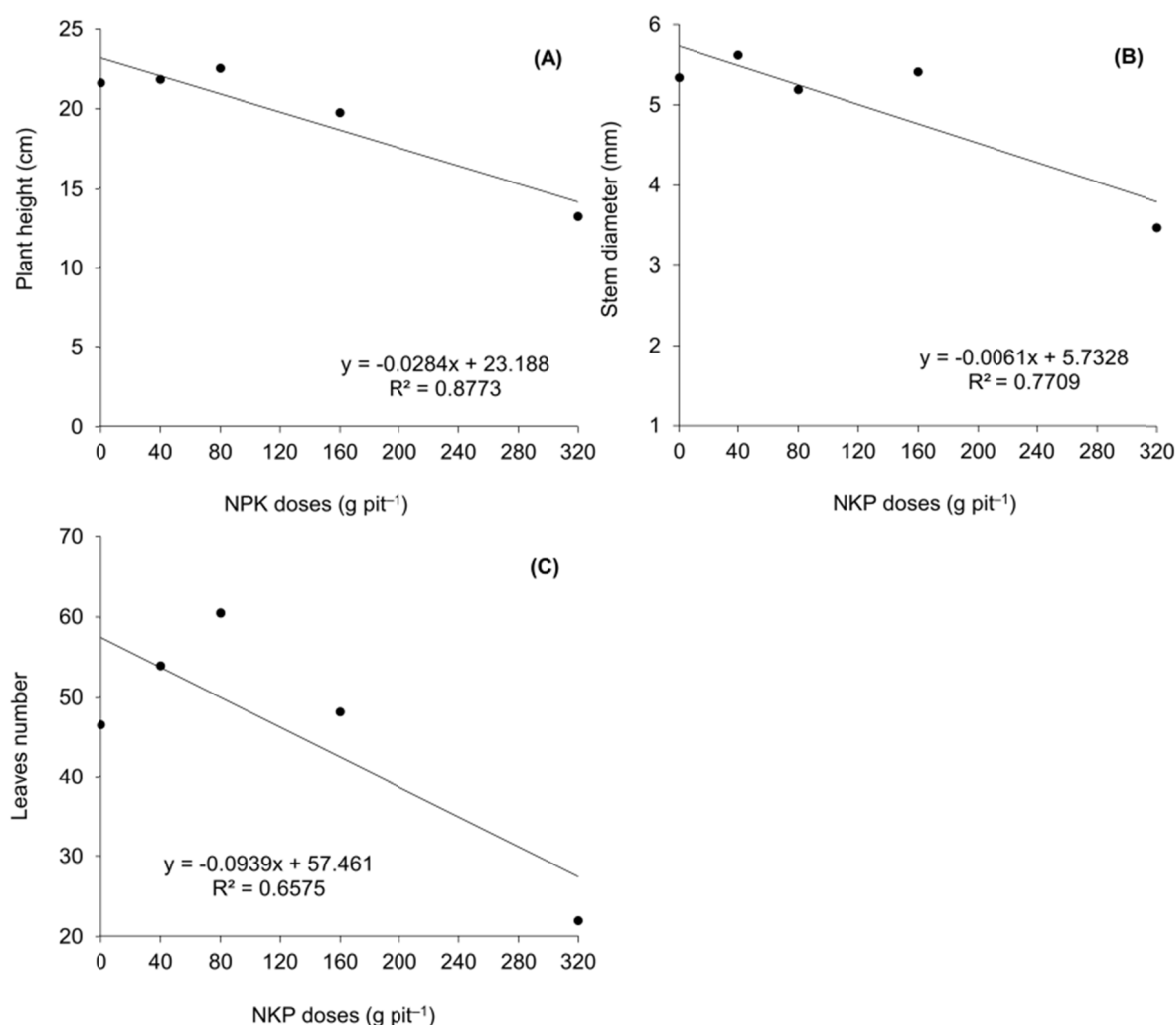


Figure 2. Effect of planting fertilization with NPK 04-14-08 on plant height (A), stem diameter (B) and leaves number (C) of *Chrysobalanus icaco*, six months after planting in an oil well base area

3.2.3 *Inga laurina*

Regarding the *Inga laurina* species, the plant height and stem diameter showed no significant difference for soil and fertilization treatments, individually. However, for the leaf number, a significant difference was observed for the two treatments (soil and fertilization). Soil and fertilization treatments showed no interaction for any of the variables. The plants that were cultivated in the clayey soil had an average of 27 leaves per plant, a higher value than that observed for the sandy soil, which was an average of 16 leaves per plant (Table 2). Regarding the doses of NPK applied at planting, the leaf number showed a linear decreasing effect (Figure 3), with a determination coefficient of 0.5465.

Table 2. Mean values of plant height, stem diameter and leaves number of *Inga laurina*, six months after planting, in relation to the type of soil in an oil well base area

Variables	Plant height (cm)		Stem diameter (mm)		Leaves number	
	Sandy	Clayish	Sandy	Clayish	Sandy	Clayish
Mean	36.55a	48.49a	6.97a	8.40a	16.15b	27.11a

Note. Means followed by the same letter horizontally do not differ at 5% probability by Tukey's test.

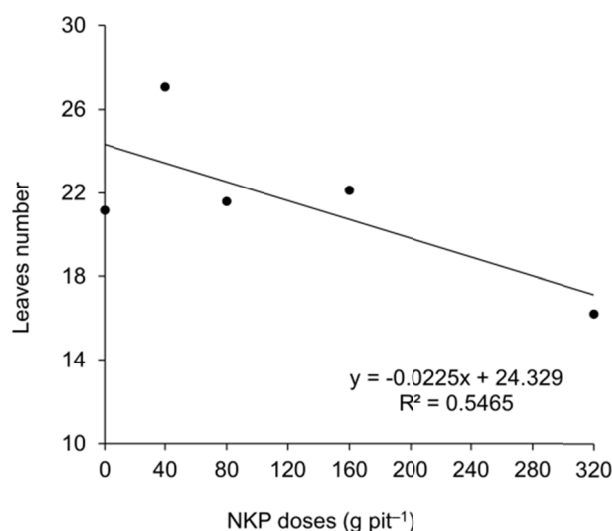


Figure 3. Effect of planting fertilization with NPK 04-14-08 on the leaves number of *Inga laurina*, six months after planting in an oil well base area

3.2.4 *Mouriri guianensis*

The variables PH, SD and LN showed no statistical difference when comparing the two soil types. However, significant responses were observed when analyzing the fertilization treatment separately for the variable leaves number. There was no significant difference for interaction between soil and fertilization treatments. The fertilization doses significantly interfered in the leaves number of the plants. The regression model that best fitted the data was the quadratic model (Figure 4). The coefficient of determination was 0.6755 and the maximum point was reached in the dose of 189.83 g pit⁻¹ of NPK, presenting a mean of 40.92 leaves per plant in this dosage.

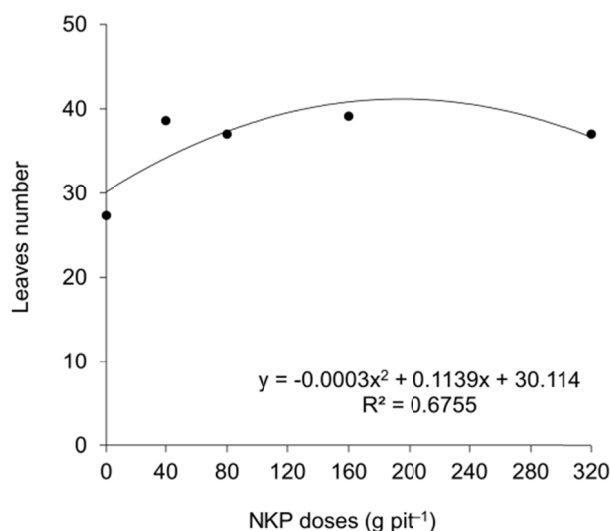


Figure 4. Effect of planting fertilization with NPK 04-14-08 on the leaves number of *Mouriri guianensis*, six months after planting in an oil well base area

3.2.5 *Garcinia brasiliensis*

The plants of *G. brasiliensis* did not show significant difference between the two soils types for the growth variables. However, the LN variable showed a significant difference in the fertilization treatment. There was no significant interaction between soil and fertilization treatments for any variable.

The descending linear regression model was the best fit to the data for the leaves number, indicating that the species *G. brasiliensis* produces a greater quantity, at the lowest NPK concentrations (Figure 5).

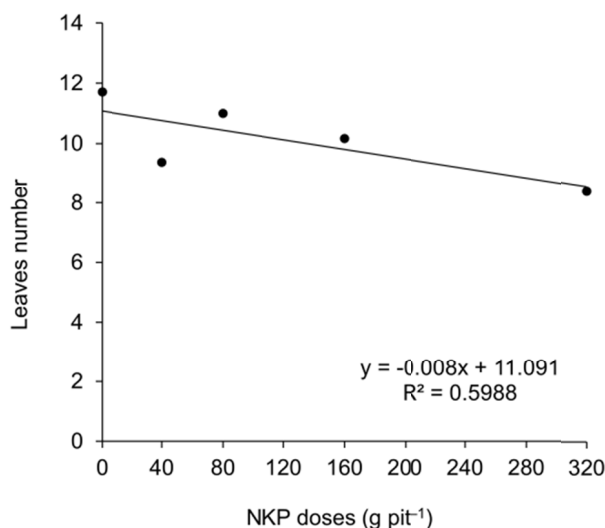


Figure 5. Effect of planting fertilization with NPK 04-14-08 on the leaves number of *Garcinia brasiliensis*, six months after planting in an oil well base area

3.3 Foliar Attributes

3.3.1 *Schinus terebinthifolius* Raddi

Regarding the leaf attributes, the *S. terebinthifolius* Raddi species showed significant results only for the variable leaf thickness (LT). The succulence (SUC), leaf mass per leaf area (LMA) and the sclerophyllia index (SI) showed no statistical difference between the treatments tested. For LT, the species showed a significant difference both for the interaction between soil and fertilization factors and for the interaction between soil and fertilization treatments. When analyzing the unfolding of the interaction for the soil factor within each fertilization level, a greater leaf thickness (0.27 mm²) was obtained for the sandy soil when 160 g pit⁻¹ of NPK was applied (Figure 6). For the other doses used in planting, the TL values showed no difference between the two soil types evaluated. However, when analyzing the interaction of fertilization within each soil level, the model that best fitted the data in the two soil types was the increasing linear model, with a determination coefficient of 0.7647 and 0.6409 for the sandy and clayey soil, respectively. A similar effect of NPK doses was observed in both soils, occurring a linear increase in leaf thickness with increasing fertilizer doses (Figure 6).

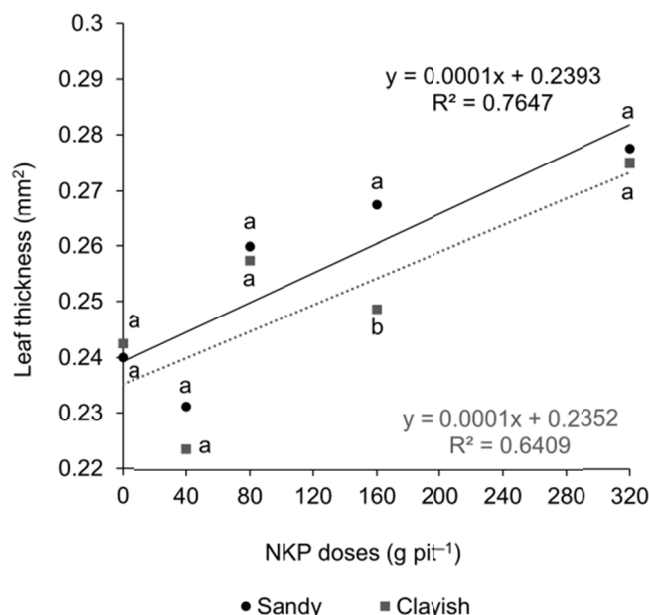


Figure 6. Unfolding of the interaction between soil factors, represented by lowercase letters (means followed by the same letter vertically do not differ at 5% probability by Tukey's test) and fertilization with NPK 04-14-08 (represented by regression) on leaf thickness of *Schinus terebinthifolius* Raddi, six months after planting in an oil well base area. Black line and gray dotted line represent sandy soil and clay soil, respectively

3.3.2 *Chrysobalanus icaco* L.

The LT and SUC leaf variables showed no significant results for the treatments tested (Table 3). The LMA and SI showed significant differences for soil and fertilization treatments separately. There was no significant interaction between the soil and fertilization factors. Considering the LMA variable, a higher value was observed when cultivated in sandy soil (Table 3). Regarding the fertilization treatment, the model that best explained the effect of doses as a function of the LMA of the plants was the decreasing linear model (R^2 0.9593) (Figure 7A). The SI presented plants with the highest value in the sandy soil. Regarding the treatment with fertilization, the regression model that adjusted to the results of the two species was the decreasing linear model, with a determination coefficient of 0.9566 (Figure 7B).

Table 3. Mean values of the soil factor for leaf thickness (LT), succulence (SUC), leaf mass per leaf area (LMA) and sclerophilia index (SI) in *Chrysobalanus icaco*, six months after planting in an oil well base area

Variables	Means	
	Sandy	Clayish
LT (mm ²)	0.31a	0.31a
SUC (g m ⁻²)	265.85a	263.67a
LMA (g m ²)	126.95a	117.27b
SI (g mm ²)	0.45a	0.41b

Note. Means followed by the same letter horizontally do not differ at 5% probability by Tukey's test.

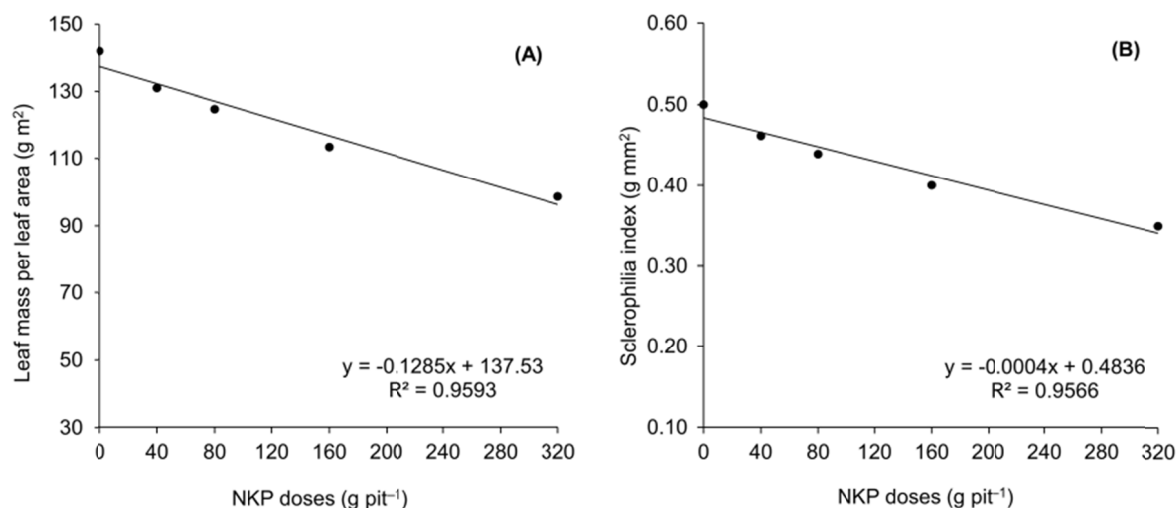


Figure 7. Effect of planting fertilization with NPK 04-14-08 in relation to leaf mass per leaf area (A) and sclerophyllia index (B) in *Chrysobalanus icaco*, six months after planting in an oil well base area

3.3.3 *Inga laurina*

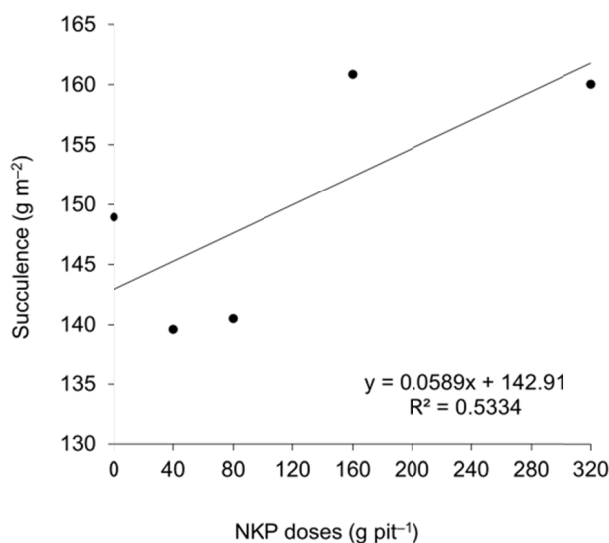


Figure 8. Effect of planting fertilization with NPK 04-14-08 in relation to succulence in *Inga laurina*, six months after planting in an oil well base area

3.3.5 *Mouriri guianensis*

In *M. guianensis*, the variables LT and leaf SUC showed no significant difference for the soil factor. Both variables showed statistical significance for the fertilization factor and also for the interaction of the soil and fertilization factors (Table 4). Leaf mass per leaf area (LMA) and sclerophyllia index (SI) showed no significant difference for the treatments tested. When analyzing the unfolding of the interaction for LT, the *M. guianensis* species presented different responses when compared with the soil factor within each fertilization level (Table 4). Plants that received doses of 40 and 320 g pit⁻¹ of NPK showed greater leaf thickness in the sandy soil; however, those that received 80 g pit⁻¹ of NPK showed greater leaf thickness in the clayey soil. It was observed that the leaf thicknesses of the plants were statistically equal in the two types of soil when considering the doses 0 and 160 g pit⁻¹ of NPK. When analyzing the fertilization factor within each type of soil, only the sandy soil data showed regression adjustment. A quadratic adjustment was observed, with a determination coefficient of 0.9950.

The minimum point was reached at a dose of 100 g pit⁻¹ of NPK, indicating a significant reduction in leaf thickness at this dosage of NPK (Figure 9A).

Table 4. Analysis of the soil factor within each fertilization level considering the average values of leaf thickness (LT) and succulence (SUC) in *Mouriri guianensis*, six months after planting in an oil well base area

Fertilization NPK 04-14-08 (g pit ⁻¹)	LT (mm ²)		SUC (g m ⁻²)	
	Sandy	Clayish	Sandy	Clayish
0	0.38a	0.39a	272.51a	288.98a
40	0.37a	0.34b	275.61a	254.45b
80	0.37b	0.41a	280.86b	313.41a
160	0.37a	0.37a	316.99a	271.47b
320	0.40a	0.38b	322.52a	286.75b
Mean	0.38	0.38	293.7	283.01

Note. Means followed by the same letter horizontally do not differ at 5% probability by Tukey's test.

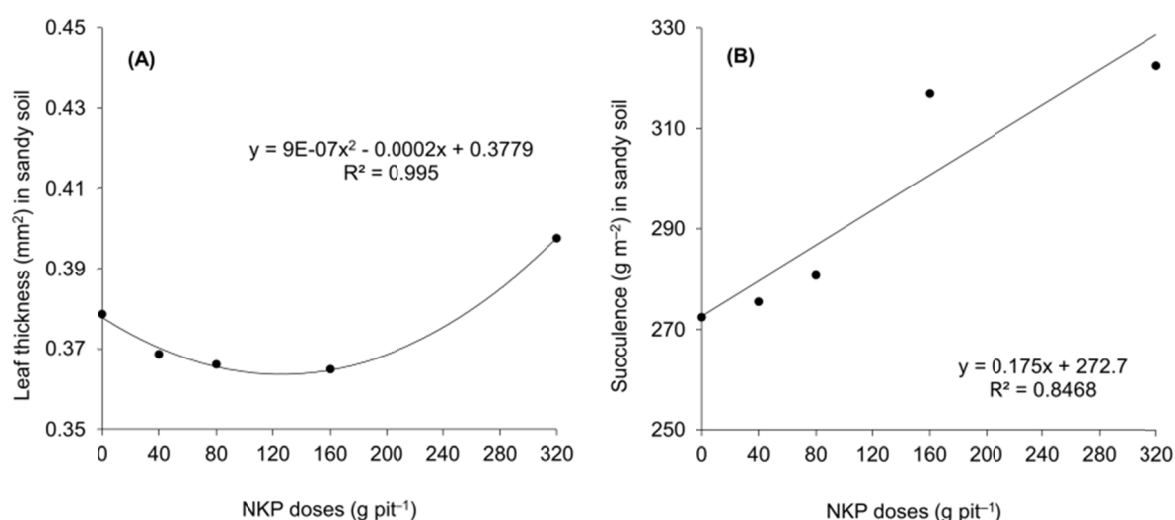


Figure 9. Analysis of the fertilization factor with NPK 04-14-08 within the sandy soil for leaf thickness (A) and succulence (B) in *Mouriri guianensis*, six months after planting in an oil well base area

When analyzing the unfolding of the interaction for leaf SUC, regarding the soil factor within each fertilization level, plants grown at doses of 40, 160 and 320 g pit⁻¹ of NPK showed higher SUC in sandy soil. However, plants that received 80 g pit⁻¹ of NPK showed higher SUC in clayey soil. Therefore, for the species that did not receive planting fertilization (0 g pit⁻¹), the SUC showed no difference between the two soil types (Table 4). When analyzing the fertilization factor within each soil type, the adjusted model was the increasing linear model, with a determination coefficient of 0.8468 for the sandy soil (Figure 9B). SUC increased with increasing doses of NPK. For clayey soil, the data did not fit any regression model.

3.3.5 *Garcinia brasiliensis*

All variables (LT, SUC, LMA and SI) showed a significant difference between treatments, since the LT and SUC showed a difference for the soil and fertilization factors separately and also for the interaction between the soil and fertilization factors. Considering the LMA and SI variables, was observed a difference for the soil factor, but fertilization was not significant. However, the interaction of soil and fertilization factors was significant. The unfolding of the interaction for leaf thickness, of the soil within each fertilization level, shows that the plants that received 0.40 and 160 g pit⁻¹ of NPK presented greater leaf thickness in the sandy soil. The 320 g pit⁻¹ dose provided an increase in LT for the clayey soil plants; however, there was no difference between the plants that received 80 g pit⁻¹ of NPK in the two soil types (Figure 10A). When analyzing the fertilization factor within each soil type, a decreasing linear adjustment was observed for the sandy soil, with a determination coefficient of

0.5042. In contrast, for the clayey soil, the adjustment was the increasing linear model, with a determination coefficient of 0.5294 (Figure 10A). These results indicate a reduction in leaf thickness for plants grown in sandy soil and an increase in LT for plants grown in clay soil at the highest doses of NPK.

When analyzing the unfolding of the interaction for the leaf SUC, regarding the soil factor within each fertilization dose, it was observed that the plants that received 40 and 320 g pit⁻¹ of NPK, presented higher SUC in the sandy soil. Plants that received 0.80 and 160 g pit⁻¹ showed no difference between the two soils types (Table 5). In the analysis of the fertilization factor within each soil type, was observed an adjustment of the quadratic model for the sandy soil, with a determination coefficient of 0.6998. The lowest SUC occurred in plants that received 121.26 g pit⁻¹ of NPK, which increased after this dosage (Figure 10B). The regression for the clayey soil had no adjustment.

Table 5. Analysis of the soil factor within each fertilization level considering the mean values of succulence (SUC) in *Garcinia brasiliensis*, six months after planting in an oil well base area

Fertilization NPK 04-14-08 (g pit ⁻¹)	SUC (g/m ⁻²)	
	Sandy	Clayish
0	196.05a	185.08a
40	230.80a	164.89b
80	197.41a	189.39a
160	175.67a	189.11a
320	268.37a	194.92b
Mean	213.66	184.68

Note. Means followed by the same letter horizontally do not differ at 5% probability by Tukey's test.

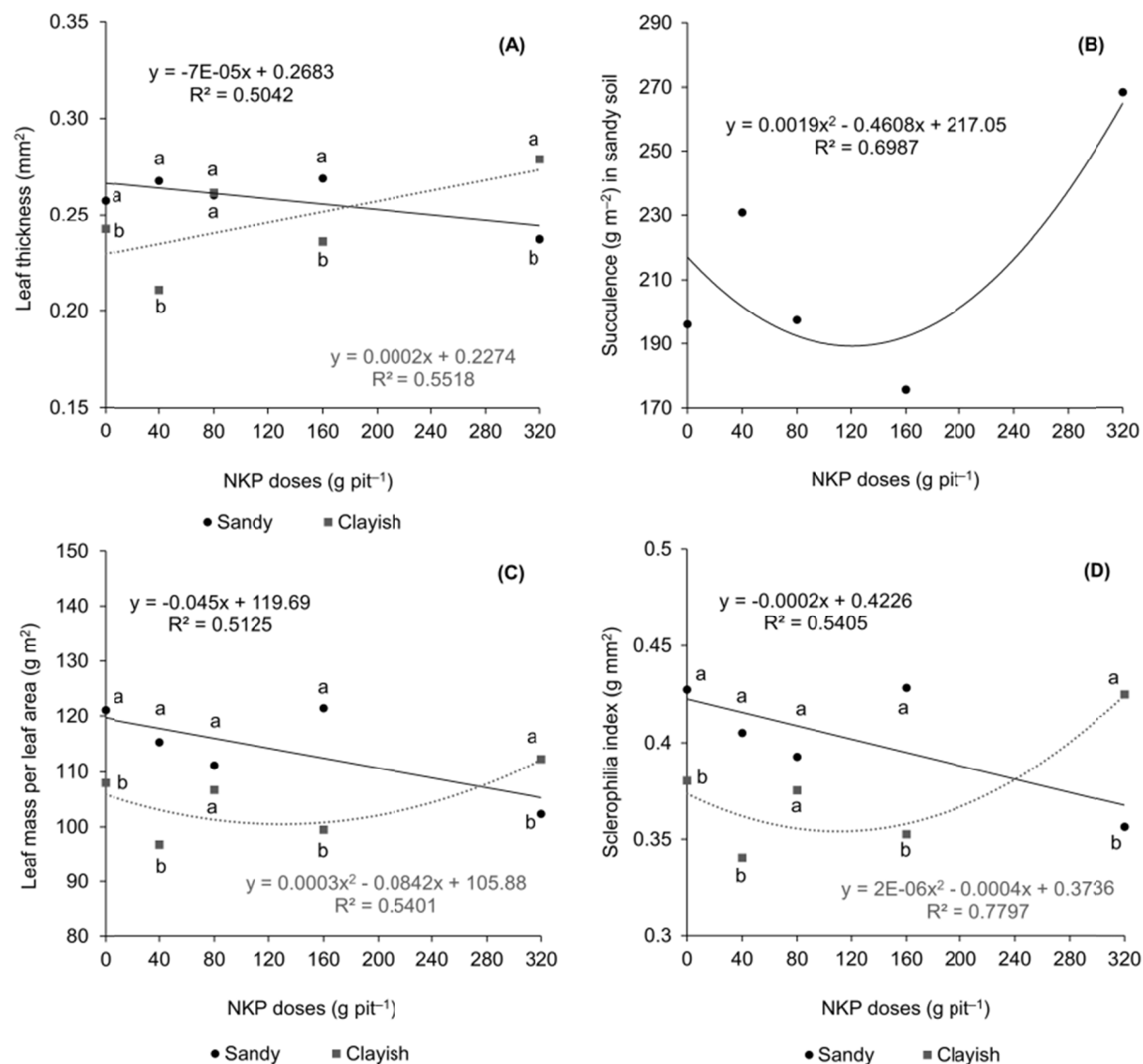


Figure 10. Unfolding of the interaction between soil factors, represented by lowercase letters (means followed by the same letter vertically do not differ at 5% probability by Tukey's test) and fertilization with NPK 04-14-08 (represented by regression) on leaf thickness (A), succulence (B), leaf mass by leaf area (C) and sclerophylla index (D) of *Garcinia brasiliensis*, six months after planting in an oil well base area. Black line and gray dotted line represent sandy soil and clay soil, respectively

Regarding the MFA variable, when analyzing the soil factor within each fertilization level, it was observed that plants cultivated with 0.40 and 160 g pit⁻¹ of NPK showed higher LMA in the sandy soil. The clay soil plants showed higher MFA when 320 g pit⁻¹ of NPK was applied. In contrast, it was observed no difference between the soil types at the dose of 80 g pit⁻¹ (Figure 10C). In the interaction of fertilization within each soil, there was a decreasing linear adjustment for the sandy soil (R^2 0.5125) and quadratic adjustment for the clay soil (R^2 0.5401). The sandy soil caused a reduction in LMA with the increase in NPK doses (Figure 10C). In the clayey soil, the lowest MFA value was observed when the plants received 140.33 g pit⁻¹ of NPK (Figure 10C).

Regarding the SI, when analyzing the soil factor within each fertilization level, it was observed that the plants that received doses of 0.40 and 160 g pit⁻¹ showed a higher SI in the sandy soil. Plants that received 320 g pit⁻¹ of NPK showed higher SI in the clayey soil; however, those that received 80 g pit⁻¹ did not differ between the two soils (Figure 10D). When analyzing the fertilization factor within each soil, was observed a decreasing linear adjustment for the sandy soil with a coefficient of determination of 0.5405 (Figure 10D), indicating a decrease in

SI with the increase in NPK doses. In the clayey soil, a quadratic polynomial adjustment with R^2 0.7797 was obtained, with a significant reduction in the SI of the plants that received $100.00 \text{ g pit}^{-1}$ (Figure 10D).

4. Discussion

4.1 *Schinus terebinthifolius* Raddi

The *S. terebinthifolius* species is highly resistant to various environmental conditions and can grow well even in poor and dry soils (Oliveira et al., 2008; Resende et al., 2015; Scheer et al., 2017; Silva et al., 2019), which explains the high survival rate observed for the species. According to Nunes et al. (2015), species considered to be pioneers, with fast growth and low soil requirements, show greater survival aptitude in degraded places. This species significantly expressed good growth when subjected to increasing of fertilization doses (Figure 1). Some authors also found high growth for *S. terebinthifolius* when subjected to fertilization with NPK (Sousa et al., 2006; Scheer et al., 2017), showing a positive response to this type of fertilization. According to Silva et al. (2018), fertilization is extremely important in the initial growth phase of native species.

After analyzing the leaf attributes, was observed an increase in LT with the increase in fertilizer doses, which may be related to the efficiency in the use of NPK by the species (Silva et al., 2018). As a pioneer species with short-lived leaves, *S. terebinthifolius* invests in less protected leaves with a higher concentration of nutrients (Coley, 1983; Poorter & Bongers, 2006). According to Coley (1983), the leaves of the pioneers have a higher concentration of nitrogen; thus, the more nutrients available in the soil, the greater the efficiency of use by the species, which provided an increase in leaf thickness. Lacher (2004) reports a relationship between the increase in dry matter and the assimilation of carbon and nitrogen by plants, since carbon not used in respiration can increase dry matter and be used to reserve or grow plants. Furtini Neto et al. (2000) consider that pioneer species have greater nutrient absorption capacity compared to other successional stages.

4.2 *Chrysobalanus icaco*

The *C. icaco* species is adapted to environments with low fertility and reflects low development when subjected to high concentrations of nutrients in the soil solution. The shortage or excess of nutrients are harmful to the development of plants, characterizing either deficiencies or toxicity, respectively, according to the limit characteristic of each species (Malavolta, 1980).

The sandy soil presents a great leaching of cations, such as Na, K, Ca and Mg along the soil profile (Werle et al., 2008; Centeno et al., 2017). However, for the plant, this fact may have been positive up to the dose of 80 g pit^{-1} because, from the supply of the dose of 160 g pit^{-1} , deaths increased with increased doses. According to Bastos (1995), *C. icaco* is a species that is easy to adapt, since it does not require high concentrations of soil nutrient for its development. It is found very close to the sea over the dunes, in open restinga and mangroves in the form of shrubs, being considered a pioneer species (Silva & Menezes, 2012).

The survival rate was even lower when the plants were grown in clay soil, compared to sandy soil. The higher plant mortality in the clayey soil/ 320 g treatment (Table 1) can be explained by the greater capacity of nutrient retention that the clayey soil has compared to the sandy, which may have caused growth inhibition due to salinity, higher than the absorption capacity of this species (Centeno et al., 2017). Silva and Corrêa (2008), using NPK fertilization to recover mined areas, also recorded low survival in *Kielmeyera lathrophytum* of only 6.7%, indicating fertilization as a probable cause of deaths. It is important to observe that during the development of the seedlings, cover fertilization was conducted with NPK 20-00-20. These fertilizations may also have contributed to the low survival rate observed for the *C. icaco* specie, considered to be less demanding in nutrients (Bastos, 1995).

The plant height, stem diameter and leaves number had a negative effect when higher doses of NPK were applied at planting, with a noticeable reduction in all variables. *C. icaco* did not respond well to the increase in chemical fertilization, showing better development among the plants that received the lowest fertilization doses. The 320 g pit^{-1} dose of NPK provided the least growth of the species, and these results are in accordance with the survival rate of the plants one year after planting (Table 1). The intrinsic combination of nutrient concentrations can express a plant's maximum yield. However, the excess of nutrients can promote a nutritional imbalance, which will affect the concentration and / or absorption of other nutrients (Fernandes et al., 2003).

Regarding the leaf attributes, the LMA of the plants was reduced due to the increase in planting fertilization (Figure 7A). According to De La Riva et al. (2016), plants with lower LMA values grow in environments with a higher concentration of nutrients and those with an elevation in this variable are observed in environments that are poorer in nutrients. The increase in LMA values found in the sandy soil for *C. icaco* may be linked to a conservative strategy in the use of resources, which provide leaves with greater longevity. Longer lasting leaves

are extremely relevant in resource-limited environments and at a high cost for the new leaves production (Wright et al., 2002).

According to the results for SI, plants grown in sandy soils showed adaptability to environmental stress conditions (Table 3). It was also observed that the treatments with the lowest concentrations of nutrients have the highest SI. The sclerophyllia index reduced with the increased fertilization doses (Figure 7B), this is the reason why is evaluated in different plant genera and has been linked to stress tolerance. The factors that influence this functional trait need further clarification, but are related to the lack of nutrients, herbivory, water stress and tolerance to cold. The high investment cost of sclerophyllia is generally associated with greater leaf longevity (Alonso-Forn et al., 2020).

4.3 *Inga laurina*

The survival rate of this species was, for most treatments, 100%. The clayey soil provided better growth for this species due to the fertility conditions of the two soils evaluated. The clayey soil has better fertility conditions than the sandy one (Donagemma et al., 2016). The growth averages (in height) obtained in the experiment six months after planting, were 36.55 and 48.49 cm for the sandy and clayey soils, respectively (Table 2). These values are within the growth range found in the literature. Moraes et al. (2013) emphasize that *I. laurina* is a non-pioneer species (initial secondary), but with a rapid growth, between 30 to 100 cm/year. It was possible to observe then an effect similar to that of *C. icaco*, whose leaf number reduced with the increase in the doses of NPK 04-14-08 applied.

Regarding leaf attributes, an increase in leaf SUC was observed with the increase in NPK doses (Figure 8). According to Medeiros et al. (2012), the accumulation of nutrients in the soil can cause salinization, which may have led to osmotic adjustment in both species. Silva et al. (2009) also observed an increase in leaf SUC in physic nut plants grown under saline stress. Trindade et al. (2006) report that the succulence allows the regulation of the concentration of salts in leaf tissues and depends directly on the absorption, transport and accumulation of ions in the leaf tissues.

4.4 *Mouriri guianensis*

The high index of initial and final survival rate observed for the species may be strongly related to the cultural treatments, which were conducted throughout the experiment, such as the rescue irrigation during the most critical period for seedling survival, ant control and also weeding practices, preventing the proliferation of weeds. According to Nunes et al. (2015), the main causes of mortality of seedlings planted in degraded areas are water stress, competition with weeds and the attack of ants, and it is important to adopt appropriate management practices for the success of planting.

Regarding plant growth, the increase in the leaves number was proportional to the fertilization up to the maximum point. Plants that did not receive planting fertilization (0 g pit⁻¹) presented a reduced number of 30 leaves (Figure 4). The leaves are of great importance for the growth of plants in the field, since they represent a larger photosynthetically active area (Melo et al., 2007). In contrast, a reduced leaf number can promote the reduction of photosynthetic activity, which may affect plant growth (Carvalho et al., 2006). Nutritional restriction is considered one of the main barriers to plant development (Lima et al., 2016); lack of nutrients can slow its growth and cause metabolic changes. In *M. guianensis*, this nutritional restriction affected the formation of new leaves visibly (Figure 4).

Regarding the leaf attributes, environments with high light and poor nutrients seemed to increase in leaf thickness (Rosado & Mattos, 2007), as was observed in plants that received 320 g pit⁻¹ of NPK in sandy soil (Table 4). For Martins et al. (2009), the reduction in leaf thickness may be attributed to the difference in the distribution and consumption of photoassimilates for leaf expansion. According to Gobbi et al. (2011), other factors such as water deficit and temperature increase can also increase leaf thickness.

In *M. guianensis*, leaf SUC increased with the increase in NPK doses, a behavior similar to that observed in the *I. laurina* species (Figure 9), which can also be related to soil salinization due to increased fertilization. Thus, as a form of protection, plants increase SUC and LT to promote regulation of the salt concentration in leaf tissues, as occurred in *M. guianensis* (Trindade et al., 2006).

In general, most of the plants grown in sandy soil showed higher leaf SUC (Table 4). This evolutionary characteristic is fundamental for the survival of the species in places of greater water scarcity. Surprisingly, little is known about the molecular regulation of leaf succulence, despite the scientific advances in this area. Information on leaf succulence is still overlooked for adapting to the water deficit and improving growth and development of plants of economic interest. Connecting functional genomics of leaf patterning with knowledge

of the evolution and ecology of succulent species will guide future research on the determination and maintenance of leaf succulence. Furthermore, the knowledge acquired from leaf succulence can be used for research with other organs, such as stems and roots (Heyduk, 2021)

4.5 *Garcinia brasiliensis*

The survival rate of the species *G. brasiliensis* presented one of the greatest variations in responses to fertilization (Table 1). According to Oliveira et al. (2015), survival index values above 60% are considered excellent for impacted environments. In general, all treatments showed a good survival rate, with the exception of the treatment with the use of 160 g pit⁻¹ of NPK in clay soil.

G. brasiliensis is a non-pioneer species and can be considered late secondary (Prado Junior et al., 2011), which is characterized by slow growth and may form small or even large trees (De Paula et al., 2004). Six months after planting, the plants showed no statistical difference for height, with the averages being 10.53 and 10.29 cm for the sandy and clay soils, respectively.

The leaves number decreased with the increase of fertilization, being approximately 23% lower in the dose of 320 g pit⁻¹ of NPK compared to zero dose. According to Poorter and Bongers (2006), late secondary species have low leaf growth, but these have a long duration. As reported by the authors, these species invest in well-protected leaves with a low content of nutrients that can decrease physical damage and herbivory.

For *G. brasiliensis*, in general, planting in sandy soil provided a reduction in LT with increasing doses. This fact can be explained by the water and nutrient retention capacity of each type of soil studied (Centeno et al., 2017). In the sandy soil, due to its high leaching, the species created an adaptive strategy to environmental pressures by increasing the LT in the lowest doses of NPK. However, in clayey soil, there was observed a tendency to increase leaf thickness with increasing doses, which also occurred in *M. guianensis*, but in sandy soil. According to Amorim and Melo Júnior (2017), the increase in leaf thickness is one of the structural characteristics of species that occupy coastal regions, which contributes to the maintenance of the water balance of plants.

Leaf thickness (LT) is an adaptive characteristic that varies depending on the stress conditions of the leaves. Greater leaf thickness can ensure greater water storage, avoiding large variations in the water potential of the plant (Rossatto & Kolb, 2013; Taiz et al., 2017). When subjected to low fertilization and sandy soil with low water retention, *G. brasiliensis* increased the leaf thickness to balance the water potential. The higher doses of fertilization used under the clayey soil caused the salinization of the soil, which also lead to increased LT responses as a plant strategy to avoid inhibition of growth and photosynthesis (Figure 10A). This behavior is in accordance with the literature (Taiz et al., 2017).

The increase in leaf succulence can be a strategy of the species in maintaining leaf hydration through water storage, protecting the plant against withering and cellular contraction (Campelo et al., 2018). Godoy and Gianoli (2013) report that the leaf succulence allows water to be stored when availability decreases and demand increases, being considered an evolutionary feature in stressful environments.

Sclerophyllia index (SI) decreased linearly with the increase of fertilization in the sandy soil. The 320g dose per pit⁻¹ of NPK favored a higher SI for the clayey soil. According to Gonçalves-Alvim et al. (2006), the sclerophyllia index can be considered as an adaptive response of plants to soils with low water retention and poor nutrients, especially nitrogen, phosphorus and potassium. Sclerophyllic plants may present leaves resistant to herbivory, low soil fertility and with structures that reduce or tolerate water loss (Sereda et al., 2016).

5. Conclusion

The survival rate was high in *S. terebinthifolius* Raddi, *I. laurina* and *M. guianensis*, whereas it was low for the species *C. icaco* when 320 g pit⁻¹ was applied. The *G. brasiliensis* species also showed a reduction in the survival rate at the dose of 160 g pit⁻¹.

S. terebinthifolius Raddi, *C. icaco*, *M. guianensis* and *G. brasiliensis* growth was not influenced by the soil type. *I. laurina* specie showed the best growth in clayey soil.

It is not recommended to use the same dose of NPK 4-14-08 for planting fertilizer in the cultivation of different native species. It is recommended to apply 219.27 to 227.25 g pit⁻¹ for *S. terebinthifolius* Raddi and 189.83 g pit⁻¹ for *M. guianensis*. Plants of the species *C. icaco*, *I. laurina* and *G. brasiliensis* developed better without fertilization for planting.

In general, the leaf attributes showed an adaptive response of the plants regarding the conditions of environmental stress to which they were submitted, showing to be a precise method to evaluate the behavior of the studied species.

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