MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS IN YOUNG PLANTS OF *Cordia trichotoma* SUBMITTED TO THE APPLICATION OF PHOSPHORUS IN THE SOIL

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ABSTRACT – The application of phosphorus (P) doses in the soil can increase the growth of native tree species of economic and environmental interest, such as *Cordia trichotoma*. Thus, this research aims to evaluate the morphological and the physiological parameters in *C. trichotoma* seedlings, cultivated in soil with increased P content. The experiment was conducted under greenhouse conditions in pots with 2.5 dm$^3$ of soil, four doses of P (0, 150, 300 and 450 mg dm$^{-3}$), and six replicates. P content in the soil, P concentrations in the leaves, morphological attributes (height, stem diameter, aerial dry matter, root dry matter, and leaf area) and physiological attributes (photosynthetic pigment content and chlorophyll a fluorescence) were determined 180 days after transplantation. The increase in the available P content in the soil allowed greater absorption of this element by the plant’s roots, increasing the concentration in the leaves, and therefore favoring the energetic metabolism of the plants. In addition, the higher influx and accumulation of P in the plant when 450 mg dm$^{-3}$ was used, increased the concentration of the photosynthetic pigments and increased the photosynthetic capacity of the *C. trichotoma* seedlings. The highest use of the light energy by photosystem II ($F_v/F_m = 0.76$) was observed in the seedlings cultivated with 450 mg dm$^{-3}$ of P, with a 95% increase when compared to seedlings without P. Thus, we observed that this tree species is demanding and responsive to the higher P content available in the soil.

Keywords: Chlorophyll a fluorescence; Louro-pardo; Phosphate fertilizer.

PARÂMETROS MORFOLÓGICOS E FISIOLÓGICOS EM PLANTAS JOVENS DE *Cordia trichotoma* SUBMETIDAS A APLICAÇÃO DE FÓSFORO NO SOLO

RESUMO – A aplicação de doses de fósforo (P) no solo pode aumentar o crescimento de espécies arbóreas nativas de interesse econômico e ambiental, como *Cordia trichotoma*. Assim, esta pesquisa tem por objetivo avaliar parâmetros morfológicos e fisiológicos em mudas de *C. trichotoma* cultivadas em solo com aumento do teor de P. O experimento foi conduzido em casa de vegetação, em vasos com 2,5 dm$^3$ de solo, com quatro doses de P (0, 150, 300 e 450 mg dm$^{-3}$) e seis repetições. O teor de P no solo, as concentrações de P nas folhas, os atributos morfológicos (altura, diâmetro do coletor, matéria seca aérea, matéria seca radicular e área foliar) e os atributos fisiológicos (teor de pigmentos fotossintéticos e fluorescência da clorofila a) foram determinados aos 180 dias após o transplante. O incremento no teor de P disponível no solo permitiu maior absorção desse elemento pelas raízes das plantas, elevando a concentração nas folhas. Isso favoreceu o metabolismo energético das plantas, proporcionando maior crescimento. Além disso, o maior influxo e acúmulo de P na planta quando utilizado 450 mg dm$^{-3}$, incrementou a concentração dos pigmentos fotossintéticos e elevou a capacidade fotossintética das
mudas de *C. trichotoma*. O maior aproveitamento da energia luminosa pelo fotossistema II \((F_v/F_m = 0,76)\) foi observado nas mudas cultivadas com 450 mg dm\(^{-3}\) de P, com aumento de 95% quando comparadas às mudas sem adição de P. Assim, percebemos que essa espécie arbórea, é exigente e responsiva em relação ao maior teor de P disponível no solo.

**Palavras-Chave:** Fluorescência da clorofila a; Louro-pardo; Adubação fosfatada.

1. INTRODUCTION

The species *Cordia trichotoma* (Vell.) Arrab. ex Steud. (louro-pardo) belonging to the Boraginaceae family is widely distributed in tropical and subtropical forests in Argentina, Bolivia, Brazil, Paraguay and Uruguay. This species is fast growing and presents wood with high economic value added, due to its quality, workability and good finish, being much used for luxury furniture and decorative coating (Berghetti et al., 2016; Cadorin et al., 2015; Coradin et al., 2011). Thus, *C. trichotoma* has the potential to be cultivated in the formation of commercial forest stands (Rossi and Sartoretto, 2014). However, the literature does not provide important information for planting, such as the adequate use of fertilizers, especially phosphates, since the majority of soils destined to forest plantations in Brazil have low availability of phosphorus (P) (Gonçalves et al., 2008a; Gonçalves et al., 2008b), because of their high adsorption affinity to functional groups of reactive particles such as Fe and Al oxides and hydroxides (Bortoluzzi et al., 2015; Fink et al., 2016).

In forestry, liming and the application of phosphate fertilizers are practices used to raise the P content available in the soil (Gonçalves et al., 2008a; Gonçalves et al., 2008b). As a result, part of the nutrient can approach the external surface of the roots and can be absorbed from the soil solution in the form of inorganic phosphate, raising its concentration in the organs of the plants (Brunetto et al., 2015). In the interior of the plants, including the woody ones, the concentration of P determines the growth and the photosynthetic activity (Warren, 2011). Plants grown in soils with higher application of phosphate fertilizers and, consequently, higher availability of P, tend to accumulate higher levels of P in the leaves (Lambers et al., 2011; Piccin et al., 2017b) and increase growth in height, diameter, leaf area and dry matter production (Crous et al., 2015; Tng et al., 2014; Zhou et al., 2017).

The productivity of plants depends mainly on photosynthesis. Plants that are deficient in P present a reduction in ADP-ATP biosynthesis, in the carboxylation/ regeneration of ribulose-1,5-biphosphate (RuBP) and in the rubisco activity, as well as a lower stomatal conductance and CO\(_2\) concentration in the substomatal chambers (Hidaka and Kitayama, 2013; Thomas et al., 2006; Warren, 2011). The damage of the photochemical reactions in the leaves can be detected by the chlorophyll \(\alpha\) fluorescence analysis, so that when there are nutritional limitations that cause stress, there is an increase in the values of initial and maximum fluorescence and reduction in the maximum quantum yield of photosystem II (Banks, 2017; Schansker et al., 2014).

Information on the responses of P fertilization and nutrient utilization in *C. trichotoma* plants grown in subtropical regions must be explored. Thus, this study aimed to evaluate morphological and physiological parameters in *C. trichotoma* seedlings cultivated in soil with increased P content.

2. MATERIALS AND METHODS

2.1 Experiment location, collection and preparation of the seeds and soil

The study was carried out from November to June in the greenhouse of the Department of Forest Sciences of the Federal University of Santa Maria (UFSM), Santa Maria, state of Rio Grande do Sul, Southern Brazil (29º43’15’’ S and 53º43’18’’ O). Seeds of *C. trichotoma* were obtained from diaspores collected in fragments of Deciduous Seasonal Forest (29º45’22’’ S and 53º34’47’’ O). After the collection, the diaspores were processed as suggested by Berghetti et al. (2015). The seeds were sown in vermiculite and packed in polypropylene trays. The trays were laid out on benches in a greenhouse. The averages of temperature and relative humidity were 25.7ºC and 82%, respectively. Irrigations were performed four times a day, for three minutes, using microsprinklers, with a flow rate of 169 L h\(^{-1}\).
At 50 days after sowing, seedlings with developed cotyledons and protophylls were transplanted into polypropylene pots containing 2.5 dm$^3$ of a Sandy Typic Hapludalf soil (Ditzler and Monger, 2017). The soil was collected in the 0.0-0.20 m layer, air dried and sieved with a 2 mm mesh. In this soil, no acidity neutralization was necessary (Table 1), because the exchangeable Ca and Mg contents were 2.7 and 1.2 cmolc dm$^{-3}$, respectively, which is considered adequate for native forest species (Bellote and Neves, 2001).

2.2 Experimental design and treatments

The experimental design was completely randomized, with six replicates. The treatments consisted of four concentrations of P$_2$O$_5$, 0 (control), 150, 300 and 450 mg P$_2$O$_5$ dm$^{-3}$ of soil. The concentrations of P$_2$O$_5$ (source: diphosphorus pentoxide), 100 mg N dm$^{-3}$ of soil (source: ammonium nitrate) and 50 mg K dm$^{-3}$ of soil (source: potassium chloride) were applied to the soil followed by immediate homogenization. The soil was packed in polypropylene pots with a capacity of 3 L. Distilled water was added in each pot to raise the field capacity to 60%. The pots were weighed daily and, when necessary, distilled water was added to maintain the soil at its field capacity, adding distilled water when soil humidity reduced 40% of that level. During the experiment a seedling of *C. trichotoma* was kept in each pot, the average temperature inside the greenhouse was 27.4ºC and the average relative humidity was 79%.

2.3 Morphological attributes and nutrient analysis in tissue and soil

At 180 days after transplantation (DAT), the plant height was measured with a millimeter ruler and the stem diameter with a digital caliper (accuracy of 0.01 mm). The aerial part of the plant was cut close to the soil surface and divided into leaves and stem. The roots were manually separated from the soil and then washed with running water. The collected leaves were distributed on white paper with millimeter scale, pressed with transparent glass plate and photographed with a digital camera with 1.4 zoom (SONY Cyber-shot, 8.1 megapixels). Afterwards, the images were treated (contrast and brightness adjustment) and processed for determination of the leaf area (LF), with the aid of Image J. The aerial part (leaves and stem) and the roots were dried in an air circulation oven forced to 70°C until constant weight, for the determination of the dry matter.

The leaves were ground in a Willey mill, sieved with a 2 mm mesh and then subjected to nitroperchloric digestion (Tedesco et al., 1995). In the extract, P was analyzed in a spectrophotometer (model SF325NM - Bel Engineering, Italy) (Tedesco et al., 1995). In addition, a soil sample was collected in each pot after cultivation. The soil was air dried, sieved with a 2 mm mesh and subjected to P extraction by the Mehlich-1 extractor (0.05 mol L$^{-1}$ HCl + 0.0125 mol L$^{-1}$ H$_2$SO$_4$). P was determined in a spectrophotometer (model SF325NM - Bel Engineering, Italy).

In addition, the agronomic efficiency [(dry weight of shoots with P - dry weight of shoots without P)/amount of P applied] was determined according to Fageria et al. (2003) in each experimental unit.

2.4 Assessment of photosynthetic pigments

For the analysis of photosynthetic pigments, fully expanded leaves of six replications per treatment were collected and frozen in liquid N2 at 180 days

| Attributes | Values |
|------------|--------|
| Clay (pipette method) (g kg$^{-1}$) | 350 |
| Sand(pipette method) (g kg$^{-1}$) | 480 |
| Silt(pipette method) (g kg$^{-1}$) | 170 |
| Organic matter (Walkley–Black method) (g kg$^{-1}$) | 25 |
| Base saturation (g kg$^{-1}$) | 157 |
| pH in water (1:1) | 4.6 |
| Available P (extracted by Mehlich$^{+}$) (mg dm$^{-3}$) | 0.7 |
| Available K (extracted by Mehlich$^{+}$) (mg dm$^{-3}$) | 32 |
| Exchangeable Ca(extracted by KCl 1 mol L$^{-1}$) (cmolcdm$^{-3}$) | 2.7 |
| Exchangeable Mg (extracted by KCl 1 mol L$^{-1}$) (cmolcdm$^{-3}$) | 1.2 |
| Exchangeable Al(extracted by KCl 1 mol L$^{-1}$) (cmolcdm$^{-3}$) | 5.7 |
| Cation exchange capacity at pH 7.0 (cmolcdm$^{-3}$) | 25.8 |
(DAT). Chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids content were analyzed according to the methodology described by Hiscox and Israelstam (1979), where 0.05 g of a fresh leaf sample were incubated at 65°C with dimethylsulfoxide (DMSO) for two hours. The concentrations of pigments were obtained after reading the absorbance in a spectrophotometer (model SF325NM - Bel Engineering, Italy), at 480, 645 and 663 nm, according to the methodology proposed by Lichtenhaller (1987).

2.5 Assessment of chlorophyll a fluorescence

The emission of chlorophyll a fluorescence was analyzed in leaves at 180 DAT, using a JUNIOR-PAM portable light-modulated fluorometer (Walz, Germany). Measurements were performed in the morning (8:00-11:00 h), using the first fully expanded leaf (Souza et al., 2013), in three plants per treatment. Previously, the leaf was adapted to the dark for 30 minutes, for measurement of the initial fluorescence (F₀). Then the sample was subjected to a pulse of saturating light (10000 umol m⁻² s⁻¹) for 0.6 s to evaluate the maximum fluorescence (Fm), while the maximum quantum yield of PSII (Fm/F₀) was evaluated using the fluorescence induction curve.

2.6 Statistical analysis

The results were tested for normality assumptions of residues and the homogeneity of variance by the Shapiro-Wilk and Bartlett test, respectively. After that, analysis of variance (ANOVA) was performed according to the model: Yᵢj = m + tᵢ + δij, where: Yᵢj is the value observed; m corresponds to the population mean; tᵢ is the effect of the treatment and δij is the effect of the random error occurring in each experimental unit. When there was a significant effect, the means were adjusted using polynomial regressions (p<0.05), or compared by the Tukey test (p≤0.05) using the statistical package SISVAR (Ferreira, 2014).

3. RESULTS

3.1 P in soil and tissue, and morphological parameters

The P contents in the soil extracted by Mehlich-1 increased in a linear way with the increase of the doses of P₂O₅ in the soil (p=0.0001) (Figure 1a). In the soil, with the application of 450 mg P₂O₅ dm⁻³ the availability of P was about 92 times higher than the content observed in the control soil (Figure 1a).

The P concentrations in the leaves increased in a quadratic manner (p = 0.0174) with the increase of the availability of this nutrient in the soil. The leaves of the plants cultivated in the soil with application of 450 mg P₂O₅ dm⁻³ had P concentration 180% higher, in comparison to the leaves of the plants cultivated in the control soil (Figure 1b).

The values of height and stem diameter of the C. trichotoma seedlings increased linearly with the increased dose of P₂O₅ applied to the soil (Figure 2a and 2b). The plants cultivated in the soil with application of 450 mg P₂O₅ dm⁻³ presented the highest averages for height (26.6 cm) and stem diameter (7.7 mm). In this condition, the values observed for height and stem diameter were 239% and 220% higher than those observed in the plants grown in the control soil (Figure 2a and 2b).

The aerial dry matter production increased linearly (Figure 2c) and the roots in a quadratic manner (Figure 2d) with the increase in the P₂O₅ concentration applied to the soil. The higher aerial dry matter production (11.6 g plant⁻¹) was observed in the plants submitted to application of 450 mg P₂O₅ dm⁻³ (Figure 2c), equivalent to an increase of 92% in relation to seedlings cultivated without fertilization. The root dry matter production in the plants cultivated in the soil with the application of 450 mg P₂O₅ dm⁻³ (10.7 g plant⁻¹) was 11 times higher than the values observed in the roots of the plants grown in the control soil.

The application of increasing doses of P₂O₅ in the soil did not alter the agronomic efficiency of C. trichotoma seedlings. However, the highest values

![Image](https://via.placeholder.com/150)
Morphological and physiological parameters in...  

(28.35 kg kg\(^{-1}\)) were observed in plants cultivated with 450 dm\(^{-3}\) of P\(_2\)O\(_5\) (Table 2).

Leaf area values increased in a quadratic manner with the P\(_2\)O\(_5\) dose (Figure 2e). The leaf area in the plants cultivated in the soil with 450 mg P\(_2\)O\(_5\) dm\(^{-3}\) was 11 times higher than that observed in the plants grown in the control soil.

3.2 Assessment of photosynthetic pigments and chlorophyll a fluorescence

The values of chlorophyll a (Chl \(\alpha\)), chlorophyll b (Chl \(b\)) and carotenoids increased in a quadratic manner with the increase in the concentration of P\(_2\)O\(_5\) in the soil (Figure 3a, 3b, and 3c). Plants grown in soils with the addition of 450 mg P\(_2\)O\(_5\) dm\(^{-3}\) had the highest values of Chl \(\alpha\), Chl \(b\) and carotenoids. On the other hand, the lowest concentrations of these photosynthetic pigments were observed in the plants grown in the soil submitted to the application of 300 mg P dm\(^{-3}\) (Figure 3a, 3b, and 3c).

### Table 2 – Agronomic efficiency of Cordia trichotoma seedlings submitted to the applications of doses of P\(_2\)O\(_5\) and cultivated for 180 days.

| Doses of P\(_2\)O\(_5\) (mg dm\(^{-3}\)) | Agronomic efficiency (kg kg\(^{-1}\)) |
|----------------------------------------|---------------------------------------|
| 150                                    | 24.58 \(*\)ns                         |
| 300                                    | 21.45                                 |
| 450                                    | 28.35                                 |
| Means                                  | 24.80                                 |
| CV (%)                                 | 37.16                                 |

\(*\) ns = F not significant at 5% probability. CV = coefficient of variation.

Chlorophyll fluorescence parameters of the C. trichotoma seedlings, initial fluorescence (\(F_o\)) and maximum fluorescence (\(F_m\)) decreased in a quadratic manner as the concentration of P\(_2\)O\(_5\) in the soil increased (Figure 3d and 3e). The values of the maximum quantum yield (\(F_v/F_m\)) of photosystem II (PSII) increased linearly with the increase in the concentration of P\(_2\)O\(_5\) applied to the soil (Figure 3f). The lowest values of \(F_o\) and \(F_m\) were observed in the leaves of the plants cultivated in the soil with the application of 450 mg P\(_2\)O\(_5\) dm\(^{-3}\) (Figure 3d and 3e). The highest \(F_v/F_m\) ratio (0.76) was observed in seedlings cultivated in the soil with the application of 450 mg P\(_2\)O\(_5\) dm\(^{-3}\). This was an increase of 95% in relation to the seedlings cultivated without the addition of P\(_2\)O\(_5\) (Figure 3f).

4. DISCUSSION

The highest values of the morphological parameters, height, stem diameter, aerial and root dry matter production observed in the plants cultivated in the soil with the application of 450 mg P\(_2\)O\(_5\) dm\(^{-3}\), are attributed to the higher P content in the soil (Figure 1a). In this condition, P was probably mainly absorbed in H\(_2\)PO\(_4\)- and HPO\(_4\)^{2-} forms (Elanchezhian et al., 2015), and part of the nutrient was transported to the aerial part and accumulated in organs, as the leaves (Figure 1b), where its increase was observed, with the increase in the dose of P\(_2\)O\(_5\) applied to the soil.

The P in the tissue stimulated plant energy metabolism, cell division and expansion (Marschner, 2012; Noack et al., 2014; Zhou et al., 2017), which reflected in the increase in the leaf area of the plants (Figure 2e). As a result of the increase in the leaf area, a higher light absorption, CO\(_2\) assimilation and,
consequently, higher photosynthetic rate occurs (Crous et al., 2015; Zambrosi et al., 2012a; Zambrosi et al., 2012b), due to the greater amount of light energy intercepted (Taiz and Zeiger, 2013).

The concentration of P in the leaves (1.48 g kg\(^{-1}\)) of the plants cultivated in the soil with 450 mg P\(_2\)O\(_5\) dm\(^{-3}\) was interpreted as high, being considered superior to the suggested ideal range for the genus Eucalyptus, which varies between 1.0 and 1.3 g kg\(^{-1}\) (Melo et al., 2016).

This is because plants grown in soils with high P content normally absorb amounts of P above their metabolic need, leading to higher P allocation in the cell vacuole (Lambers et al., 2011; Noack et al., 2014; Veneklaas et al., 2012). On the other hand, the concentration of P in the leaves of the plants cultivated in the control soil (0.53 g kg\(^{-1}\)), can be interpreted as low (Melo et al., 2016), proving insufficient to provide to plant metabolism suitable conditions for satisfactory growth.

With the increase in the P content available in the soil, there is a greater supply of this element to the roots of the plants and probably greater absorption (Piccin et al., 2017a). A large part of the P absorbed is transported and accumulated in the leaves of the plants (Veneklaas et al., 2012), where it provides increased energy metabolism, increased cell division (Marschner, 2012), stomatal conductance (Warren, 2011) and, consequently, increased synthesis of photosynthetic pigments (Jiang et al., 2009). Increasing concentrations of chlorophyll \(\alpha\) and \(b\) and carotenoids in the leaves promotes greater absorption and capture of light in different regions of the spectrum in the early stages of the photosynthetic process (Taiz and Zeiger, 2013). Thus, a higher resonance energy transfer occurs from the antenna complexes to the reaction centers, where the energy can be used for the photochemical reactions responsible for biomass production (Taiz and Zeiger, 2013).

The lowest index of photochemical \((F_v/F_m=172.5)\) and dissipation \((F_m=565.5)\) energy losses of the PSII antenna complex observed in the seedlings cultivated in the soil with 450 mg P\(_2\)O\(_5\) dm\(^{-3}\) (Figure 3d and 3e), indicates that in this condition, the seedlings do not present damage in the reaction center of the PSII and have high excitation energy transfer from the light collecting system to the reaction center (Schansker et al., 2014; Stirbet and Govindjee, 2011).

This is in agreement with the data on the maximum quantum yield \((F_v/F_m)\) of the PSII (Figure 3f). The greater use \((F_v/F_m=0.76)\) of the light energy of PSII indicates that most of the light energy is being directed to the photochemical stage of photosynthesis, rather than being lost by fluorescence of chlorophyll \(\alpha\) (Baker, 2008).

According to Araújo and Deminicis (2009), healthy plants should have an \(F_v/F_m\) ratio between 0.75 and 0.85. Thus, for \(C.\) trichotoma, the values close to 0.76 (Figure 3f) can be considered good predictors of growth, because under these conditions the seedlings showed higher growth and dry matter production. Similar results were observed by Turchetto et al. (2016) studying the performance of native tree species, including \(C.\) trichotoma, in nursery.

The reduction of \(F_v/F_m\) observed in the seedlings grown in the control soil, characterizes a state of photoinhibition in the plants (Araújo and Deminicis, 2009), and because of this, a smaller amount of energy absorbed by the plant through the antenna complex is...
used to reduce carbon and produce dry matter, which helps to explain the low dry matter production in this condition of low P availability in the soil (Tiecher et al., 2016).

In this study, the highest values of agronomic efficiency observed at the 450 mg dm\(^{-3}\) dose of P\(_{2}O_{5}\) occurred mainly because of the larger leaf area and aerial dry matter production of *Cordia trichotoma* seedlings (Figure 1). A similar behavior was also observed by Magalhães et al. (2017) in *Eucalyptus urograndis* seedlings where the highest agronomic efficiency values occurred in the higher doses of different phosphate fertilizers.

Besides that, the addition of P\(_{2}O_{5}\) in the soil increased the availability of P yielding *C. trichotoma* seedlings with higher internal concentration of P. Thus, the plants cultivated at the concentration of 450 mg P\(_{2}O_{5}\) dm\(^{-3}\) of soil showed the highest values for the morphological parameters height, stem diameter, aerial and root dry matter. This is consistent with the results observed by authors who studied the effects of different P levels on different forest species (Wu et al., 2011; Yang et al., 2014; Yao et al., 2011).

In addition, the photosynthetic pigments and the chlorophyll a emission also presented the best values in the plants cultivated in the soil with higher P availability.

Thus, it is believed that although *C. trichotoma* plants tolerate low levels of P in the soil, they are able to respond positively to phosphate fertilization with higher growth and dry matter production.

5. CONCLUSIONS

The seedlings of *Cordia trichotoma* proved to be responsive to the increase in the P content available in the soil. The application of the highest concentration of P (450 mg P\(_{2}O_{5}\) dm\(^{-3}\)) stimulates higher growth in height, stem diameter, aerial and root dry matter and leaf area. The seedlings grown in the soil with 450 mg P\(_{2}O_{5}\) dm\(^{-3}\) presented the highest levels of photosynthetic pigments, greater use of light energy by PSII and lower rate of energy loss due to fluorescence.

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