

## THE INFLUENCE OF FERTILIZER TYPE AND *Trichoderma harzianum* INOCULATION ON THE GROWTH AND PHYSIOLOGY OF YOUNG PLANTS OF *Cordia americana*

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### Resumo

*Influência de diferentes fertilizantes e inoculação com Trichoderma harzianum no crescimento e fisiologia de plantas jovens de Cordia americana.* A presente pesquisa objetivou verificar a influência do uso de diferentes tipos de fertilizantes, associado à inoculação com *Trichoderma harzianum* no crescimento e aspectos fisiológicos de plantas jovens de *Cordia americana*. O delineamento experimental utilizado foi o inteiramente casualizado, com seis repetições, em esquema fatorial 5 x 2, sendo cinco diferentes tipos de fertilizantes e a inoculação com *T. harzianum* (presença ou ausência). Aos 180 dias após o plantio mensurou-se os atributos altura da parte aérea, diâmetro do coleto, massa seca foliar, massa seca radicular, massa seca do caule, massa seca total, área foliar, índice de clorofila Falker *a* e *b* e fluorescência da clorofila *a*. A utilização do fungo *T. harzianum* associado com o uso de fertilizante de liberação controlada ou organominerais a base de dejetos mostrou-se eficaz para um maior crescimento de plantas jovens de *C. americana* em condições similares às de campo. O uso de fertilizante de liberação controlada como adubação de base teve influência positiva sobre todas as variáveis avaliadas, sendo eficaz para maximizar o desenvolvimento inicial de plantas de *C. americana*. Fertilizantes granulados a base de dejetos suínos potencializaram o crescimento e a eficiência fotossintética, confirmando ser alternativa viável como adubação para o cultivo de *C. americana*.

*Palavras-chave:* Morfofisiologia, adubação de base, bioinsumos, suinocultura, espécies florestais nativas.

### Abstract

In this study, we sought to determine the influence of combined treatments with different types of fertilizer and inoculation of the fungus *Trichoderma harzianum* on the growth and physiological characteristics of young plants of *Cordia americana*. To this end, we adopted a completely randomized experimental design, comprising six replications in a 5 x 2 factorial scheme, to assess the effects of five different types of fertilizer applied with or without *T. harzianum* inoculation. At 180 days post-planting, we performed measurements of plant height, stem diameter, leaf dry mass, root dry mass, stem dry mass, total dry mass, leaf area, Falker's chlorophyll index, and chlorophyll fluorescence. It was found that the combined application of *T. harzianum* and a controlled-release fertilizer or manure-based organominerals was effective in enhancing the growth of *C. americana* plants under conditions similar to those in the field. The use of controlled-release fertilizer as a base fertilization was found to have a positive influence on all evaluated variables and was effective in maximizing the initial development of *C. americana* plants. Granulated fertilizers derived from swine manure were established to promote plant growth and photosynthetic efficiency, thereby confirming their efficacy as a fertilizer for the cultivation of *C. americana*.

*Keywords:* Morphophysiology, base fertilization, bioinputs, swine production, native forest species

## INTRODUCTION

The Atlantic Forest Biome contains a high diversity of plant species, among which, *Cordia americana* (L.) Gottschling, & J.S. Mill (commonly referred to as guajuvira) in the family Boraginaceae (CARVALHO, 2003) is naturally distributed in Argentina, Paraguay, Bolivia, and Brazil (TROPICOS, 2022), wherein it plays a key role in the development of the southernmost part of this biome.

*C. americana* is particularly noted for its ecological and economic importance, and is recommended for the restoration of degraded areas and commercial reforestation, as well as for the use of its timber in civil construction, outdoor work, and luxury furniture production (CARVALHO, 2003). In addition, this species has been established to have medicinal application, with its leaves being widely used in traditional medicine in southern Brazil, on account of their anti-inflammatory properties and wound-healing activities (GELLER *et al.*, 2010).

In a silvicultural context, the growth and quality of plants is directly associated with nutrient availability (ROS *et al.*, 2018), and a deficiency in nutrients is among the primary factors limiting the development of forest species. Generally, however, comparatively little detailed information is available regarding the nutritional requirements of native tree species (GONÇALVES *et al.*, 2013). In Brazil, mineral fertilizers with immediate release, based on nitrogen, phosphorus, and potassium, are the most commonly used, which can be attributed to their practicality and availability, as well as being a source of the main macronutrients required by plants during the initial stages of growth.

As an approach to enhancing soil nutrient capacity and, consequently, reducing the costs associated with chemical fertilizers, conventional fertilization regimes are often supplemented with the addition of organic residues (VIEIRA, 2022). In this regard, the pig farming sector has undergone a notable expansion in recent years, with Brazil being the fourth largest global pork producer (USDA, 2019). However, despite contributing to economic development, large-scale production generates significant volumes of waste, which is considered a primary source of freshwater pollution worldwide (ARAÚJO *et al.*, 2019). The solid waste generated by pig farming can, nevertheless, be put to profitable use as an excellent alternative for the production of organic and mineral fertilizers.

Such controlled-release fertilizers have contributed to maximizing the development of forest species under nursery conditions (MEZZOMO *et al.*, 2018; GRIEBELER *et al.*, 2023), and are considered suitable for optimizing plant growth. The main advantage of such fertilizers is the gradual release of nutrients to the plant (GUO *et al.*, 2017), which prevents damage to root systems caused by the accumulation of nutrients to toxic concentrations and reduces leaching-related losses.

In addition to these developments in fertilizer formulation, fungi of the genus *Trichoderma* have been used to enhance the growth and development of agricultural and forest species (AMARAL *et al.*, 2017; GRIEBELER *et al.*, 2021). The beneficial effects observed in plants inoculated with different isolates of *Trichoderma* species are generally associated with an enhanced potential for the solubilization and absorption of nutrients within the rhizosphere (SOLDAN *et al.*, 2018) and the stimulation of plant cell multiplication via the production of indole-3-acetic acid (CHAGAS *et al.*, 2016).

Given the aforementioned considerations, in this study, we sought to investigate the effects of different types of fertilizers combined with the inoculation of *Trichoderma harzianum* Rifai on the growth, chlorophyll index, and chlorophyll *a* fluorescence of young *Cordia americana* plants.

## MATERIALS AND METHODS

### Plant material and experimental design

The study was carried out between the months of January and July 2021 at the Viveiro Florestal of the Federal University of Santa Maria, *Campus* de Frederico Westphalen (27°23'46"S, 53°25'32"W), in the municipality of Frederico Westphalen, Rio Grande do Sul, Brazil. According to the Köppen classification, the climate of the region is a humid subtropical Cfa type, with hot summers, maximum temperatures equal to or greater than 22 °C, and minimum temperatures of between -3 and 18 °C in the coldest months. The average annual precipitation is 1,900 – 2,200 mm (ALVARES *et al.*, 2013).

For the purposes of this study, we adopted a completely randomized experimental design comprising six replications in a 5 × 2 factorial scheme, which was used to assess treatments with five different types of fertilizers with and without the inoculation of *T. harzianum*. Experimental units comprised 11 L polypropylene vessels filled with a Red Oxisol, which had initially been sieved through a 6 mm mesh. The chemical characteristics of the soil were determined at the Soil Laboratory of the Universidade Regional Integrada, Frederico Westphalen.

The fertilizers used were as follows: 1) Control (without the addition of fertilizer); 2) Controlled-release fertilizer (CRF: NPK 16:8:12); 3) Traditional mineral fertilizer (TMF: NPK 20:10:15); 4) Organic and mineral fertilizer (OMF: NPK 8:4:6), obtained as mixture of organic fertilizer (OF) from the solid fraction of pig farming wastewater and TMF; and 5) Soil-remineralizing organic fertilizer (SROF: NPK 8:4:6), formulated by mixing OF with a soil-remineralizing agent (basalt powder) and TMF. The total amount of nutrients (NPK) was the same for all treatments.

The CRF used in this study was Basacote®Plus 9M, in which the fertilizer is coated with a semi-permeable elastic polymer that facilitates the gradual release of nutrients over an extended period of up to 9 months. The organic fertilizer (OF) was derived from a Swine Farming Wastewater Treatment System (Sistars), located in the swine farming sector of UFSM, Frederico Westphalen *Campus*, RS. The solid fraction, separated by settling and dewatering, along with the solid fraction separated by screening, was composted for 45 days. The composted fraction was subsequently screened and granulated. The soil-remineralizer (basalt powder) was obtained by screening crushed basaltic rock through a 0.6 mm mesh (BARROS *et al.*, 2021).

The *Trichoderma* inoculum used consisted of a commercial product formulated with *T. harzianum* (StimuControl®), with a concentration of  $1 \times 10^9$  CFU mL<sup>-1</sup>. According to the manufacturer's instructions, this

product can be used to control soil fungi and as a plant growth stimulant. The concentrated fungal culture was diluted in 1000 mL distilled water (proportion 1:1 v/v), resulting in a final spore concentration of  $5 \times 10^8$  CFU mL<sup>-1</sup>. Inoculation was performed in two stages, with 6 mL of the spore suspension being applied around the seedling clod at the time of planting seedlings in pots, and a further 6 mL was applied 15 days after planting, following a methodology adapted from that described by Griebeler *et al.* (2021).

For plant cultivation, a thin layer of gravel was initially placed in the bottom of each pot to facilitate drainage, which was overlaid with soil (with or without fertilization). Subsequently, the pots were placed in a greenhouse where they were irrigated up to 100% field capacity. Seedlings were then planted and inoculated. At the time of planting, the seedlings were on average 40.8 cm in height with a stem diameter of 8.0 mm. Throughout the study, automated daily irrigation was performed using microsprinklers, providing a water depth of 5 mm day<sup>-1</sup>.

### Morphological attributes

Plant growth assessments were conducted at the time of seedling plantation and subsequently at 30, 60, 90, 120, 150, and 180 days post-planting. The aboveground height was measured using a graduated ruler from the plant collar to the stem apex, and stem diameter was measured at ground level using a digital caliper with a precision of 0.01 mm.

Dry mass allocation was evaluated at the end of the experiment (180 days post-planting). To determine the aboveground dry mass (ADM) and root dry mass (RDM), the plants were separated into aboveground and root systems, with the latter washed under running water using sieves to remove the adhered substrate. Subsequently, the material was placed in Kraft paper packaging and dried in a forced air circulation oven at a temperature of  $65 \pm 5^\circ\text{C}$  until obtaining a constant weight. The dry mass was measured in grams using an analytical balance (precision 0.01 g). For leaf area (LA) determinations, 20 leaves were selected from each plant and distributed on a scanner with a reference scale. Images were processed using ImageJ<sup>®</sup> software.

### Physiological attributes

The chlorophyll index of plant leaves was determined with a portable meter (CF 1030; ClorofiLOG, Falker Automação Agrícola, Brazil) using expanded leaves from the upper third of the plant. One leaf per plant was analyzed (two readings per leaf, one on each side of the central vein). The results were expressed in terms of the Falker Chlorophyll Index (ICFa and ICFb), which was calculated by considering the combination of the analyzed light wavelengths.

Chlorophyll *a* fluorescence was analyzed 180 days after planting using a pulse-modulated fluorometer (Junior-Pam Chlorophyll Fluorometer; Walz Mess-und-Regeltechnik). The evaluations were performed on sunny days. Initially, a fully expanded leaf from the middle third of plants was dark-adapted for 30 min using aluminum foil. Subsequently, readings were taken between 08:00 and 11:30 to obtain values for the initial fluorescence ( $F_0$ ), variable fluorescence/maximum fluorescence ratio ( $F_v/F_m$ ), electron transport rate (ETR), and effective quantum yield of PSII [ $Y_{(II)}$ ]. The leaves were then subjected to a saturating light pulse ( $10,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 0.6 s, followed by a determination of maximum fluorescence ( $F_m$ ). The maximum quantum yield of PSII ( $F_v/F_m$ ) was calculated as the ratio of variable fluorescence ( $F_v = F_m - F_0$ ) to maximum fluorescence.

### Statistical analysis

Prior to analysis of the data, the normality of residuals and homogeneity of variance were initially assessed using the Shapiro–Wilk and Bartlett tests, respectively. Analyses were performed using a factorial analysis of variance (ANOVA). Additionally, when a significant difference was identified, the means were compared using Tukey's test with a 5% probability of error ( $p < 0.05$ ). Analyses were conducted using RStudio software (R CORE TEAM, 2018) and SigmaPlot version 12.0.

## RESULTS

Data analysis revealed a significant interaction between the study factors for the attributes height ( $p=0.0013$ ) and stem dry mass ( $p=0.0038$ ). In terms of *C. americana* mean plant height (H), we found that the tallest plants were obtained following application of the base fertilizer supplemented with controlled-release fertilizer (CRF), regardless of inoculation with *T. harzianum*. In contrast, when using the organic and mineral fertilizer (OMF) and soil-remineralizing organic fertilizer (SROF), the best response was obtained for plants cultivated in the presence of *T. harzianum* (Figure 1a).

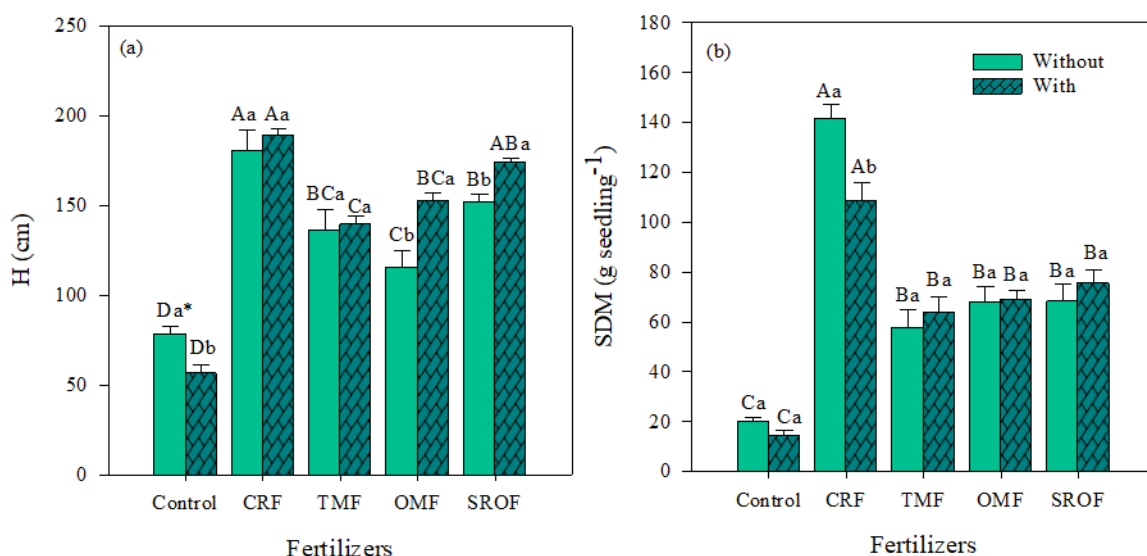


Figure 1. The effects of using different types of fertilizers with and without *Trichoderma harzianum* inoculation on the height (a) and stem dry mass (b) of *Cordia americana* plants at 180 days after transplanting. Control (without basal fertilization), Controlled-release fertilizer (CRF), Traditional mineral fertilizer (TMF), Organic and mineral fertilizer (OMF), Soil-remineralizing organic fertilizer (SROF). \*Uppercase letters above bars indicate comparison of different fertilizers for each *T. harzianum* level, and lowercase letters above bars indicate comparison of inoculation with *T. harzianum* at each fertilizer level, as assessed using Tukey's test at a 5% probability of error. Vertical bars indicate mean  $\pm$  standard error values.

Figura 1. Efeito do uso de diferentes tipos de fertilizantes com e sem inoculação de *Trichoderma harzianum* sobre a altura (a) e massa seca do caule (b) de plantas de *Cordia americana* aos 180 dias após o transplante. Controle (sem adubação de base), Fertilizante de liberação controlada (FLC), Fertilizante mineral tradicional (FMT), Fertilizante organomineral (FOM), Fertilizante organomineral remineralizador do solo (FOMRS). \*Letras maiúsculas indicam comparação dos diferentes fertilizantes para cada nível de *T. harzianum*, letras minúsculas indicam a comparação da inoculação com *T. harzianum* em cada nível de fertilizante, pelo teste Tukey a 5% de probabilidade de erro. Barras verticais indicam a média  $\pm$  erro padrão.

With regards to the allocation of stem dry mass (SDM), we found that plants cultivated in soil fertilized with CRF and without the inoculation of *T. harzianum* showed the best results, and were approximately 600% taller than plants receiving the control treatment (without fertilization). Contrastingly, we detected no significant differences in the stem dry masses of *C. americana* plants fertilized with organic and mineral fertilizers derived from pig waste (OMF and SROF) or TMF (Figure 1b).

In addition, we detected significant isolated effects of different types of fertilizer on stem diameter ( $p < 0.0001$ ), dry foliar mass ( $p < 0.0001$ ), root dry mass ( $p < 0.0001$ ), total dry mass ( $p < 0.0001$ ), and leaf area ( $p < 0.0001$ ). *Cordia americana* plants obtained a higher mean stem diameter (SDM) when treated with CRF, OMF, or SROF (Figure 2a). Compared with plants treated with TMF, we recorded 28% and 30% larger SDM in those plants receiving fertilization with OMF and SROF, respectively.

With respect to dry foliar mass (DFM), we found that the application of CRF resulted in the highest average value, which was 492% higher than that of control *C. americana* plants that received no exogenous fertilization. Contrastingly, we failed to detect any significant differences in the allocation of dry foliar mass (DFM) to *C. americana* plants fertilized with OMF, SROF, or TMF (Figure 2b). In the case of the dry mass of roots (DMR) and total dry mass (TDM), *C. americana* plants were established to perform better following the application of CRF and SROF (Figure 2c and 2d).

Similar to dry foliar mass (DFM), we found that compared with other treatments, plants cultivated with CRF had higher average values for leaf area (LA) (Figure 2e), among which, we recorded a 483% increase compared with that measured for plants that did not receive any type of fertilization.

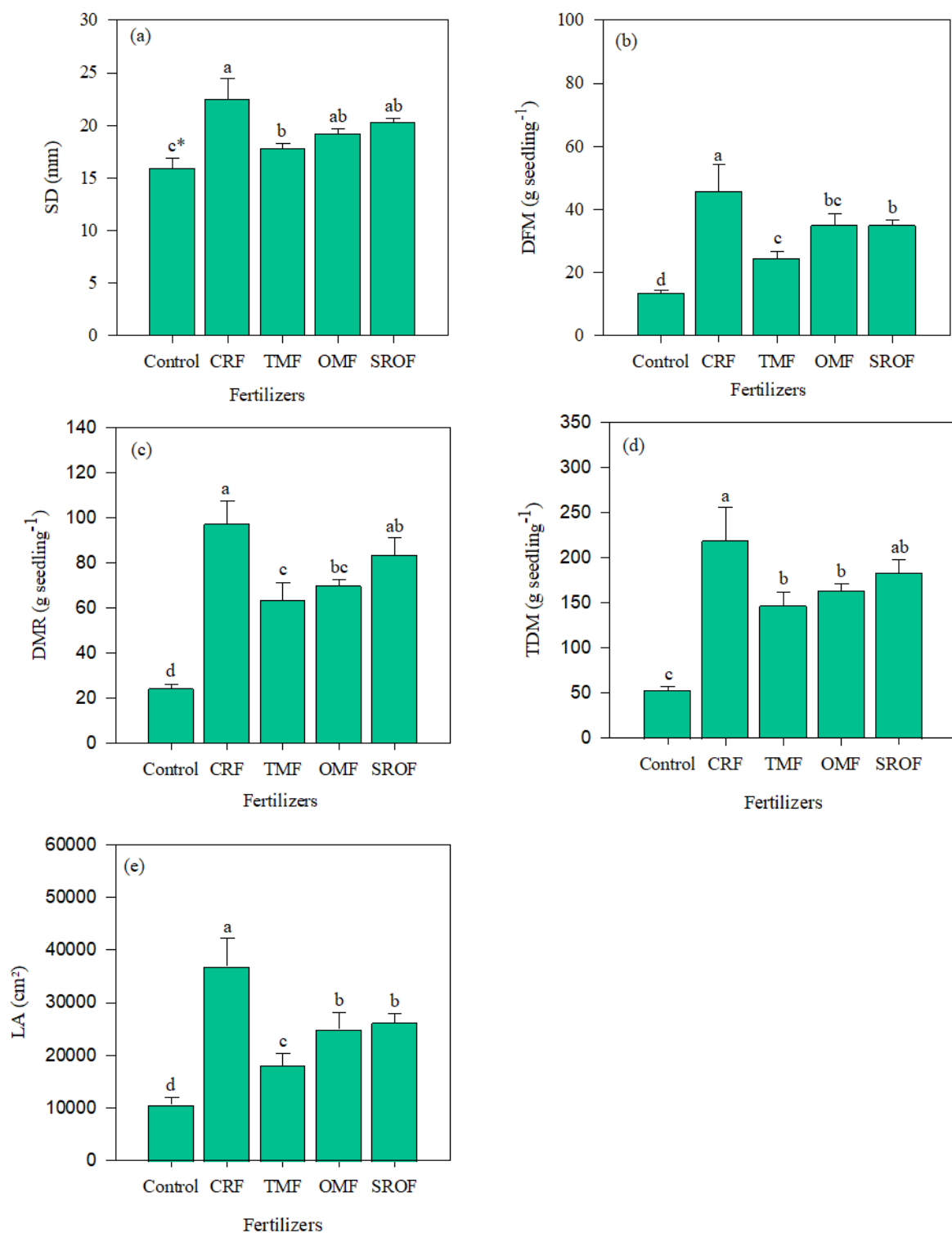


Figure 2. The effects of using different types of fertilizers on the stem diameter (a), leaf dry mass (b), root dry mass (c), total dry mass (d), and leaf area (e) of *Cordia americana* plants at 180 days after transplanting. Control (without basal fertilization), Controlled-release fertilizer (CRF), Traditional mineral fertilizer (TMF), Organic and mineral fertilizer (OMF), Soil-remineralizing organic fertilizer (SROF). \*Comparison of means using Tukey's test at a 5% probability of error. Vertical bars indicate mean  $\pm$  standard error values.



Figura 2. Efeito do uso de diferentes tipos de fertilizantes sobre o diâmetro do coleto (a), massa seca foliar (b), massa seca da raiz (c), massa seca total (d), e para a área foliar (e), de plantas de *C. americana* aos 180 dias após o transplante. Controle (sem adubação de base), Fertilizante de liberação controlada (FLC), Fertilizante mineral tradicional (FMT), Fertilizante organomineral (FOM), Fertilizante organomineral remineralizador do solo (FOMRS). \*Comparação de médias pelo teste Tukey a 5% de probabilidade de erro. Barras verticais indicam a média  $\pm$  erro padrão.

In terms of the evaluated physiological attributes, we established that the type of fertilization received had a significant effect on the ICFa ( $p < 0.0003$ ) and ICFb ( $p < 0.0001$ ) indices of *C. americana* plants, with the most pronounced effects being detected in those plants receiving the CRF treatment (Figure 3).

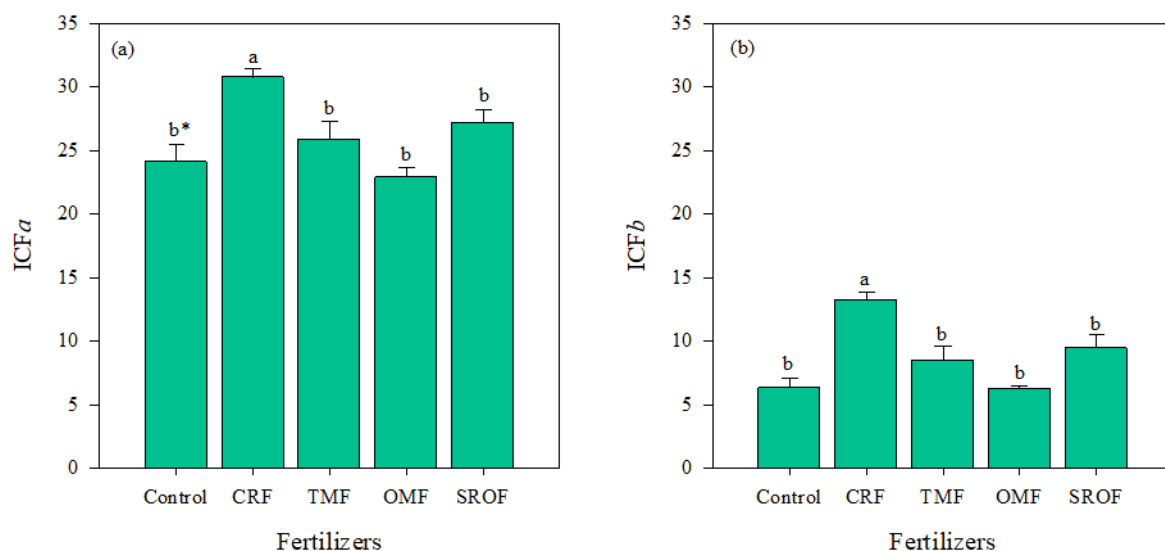


Figure 3. The effects of using different types of fertilizers on the Falker Chlorophyll Indices ICFa (a) and ICFb (b) of *Cordia americana* plants at 180 days after transplanting. Control (without basal fertilization), Controlled-release fertilizer (CRF), Traditional mineral fertilizer (TMF), Organic and mineral fertilizer (OMF), Soil-remineralizing organic fertilizer (SROF). \*Comparison of means using Tukey's test at a 5% probability of error. Vertical bars indicate mean  $\pm$  standard error values.

Figura 3. Efeito do uso de diferentes tipos de fertilizantes sobre o Índice de Clorofila Falker (ICFa e ICFb), de plantas de *C. americana* aos 180 dias após o transplante. Controle (sem adubação de base), Fertilizante de liberação controlada (FLC), Fertilizante mineral tradicional (FMT), Fertilizante organomineral (FOM), Fertilizante organomineral remineralizador do solo (FOMRS). \*Comparação de médias pelo teste Tukey a 5% de probabilidade de erro. Barras verticais indicam a média  $\pm$  erro padrão.

With respect to the fluorescence of chlorophyll *a*, the use of different fertilizers had significant effects on  $F_v/F_m$  ( $p < 0.0001$ ),  $Y_{(II)}$  ( $p < 0.0001$ ),  $F_0$  ( $p < 0.0003$ ), and ETR ( $p < 0.0001$ ). In terms of  $F_v/F_m$ , we recorded the highest values of 0.670, 0.652, and 0.644 in plants treated with CRF, OMF, and SROF fertilizers, respectively (Figure 4a), whereas fertilization with OMF and CRF resulted in plants with the highest average  $Y_{(II)}$  values (Figure 4b). Furthermore, compared with the non-fertilized controls plants, we recorded a 132.35% increase in the  $F_0$  variable of plants receiving the SROF treatment (Figure 4c).

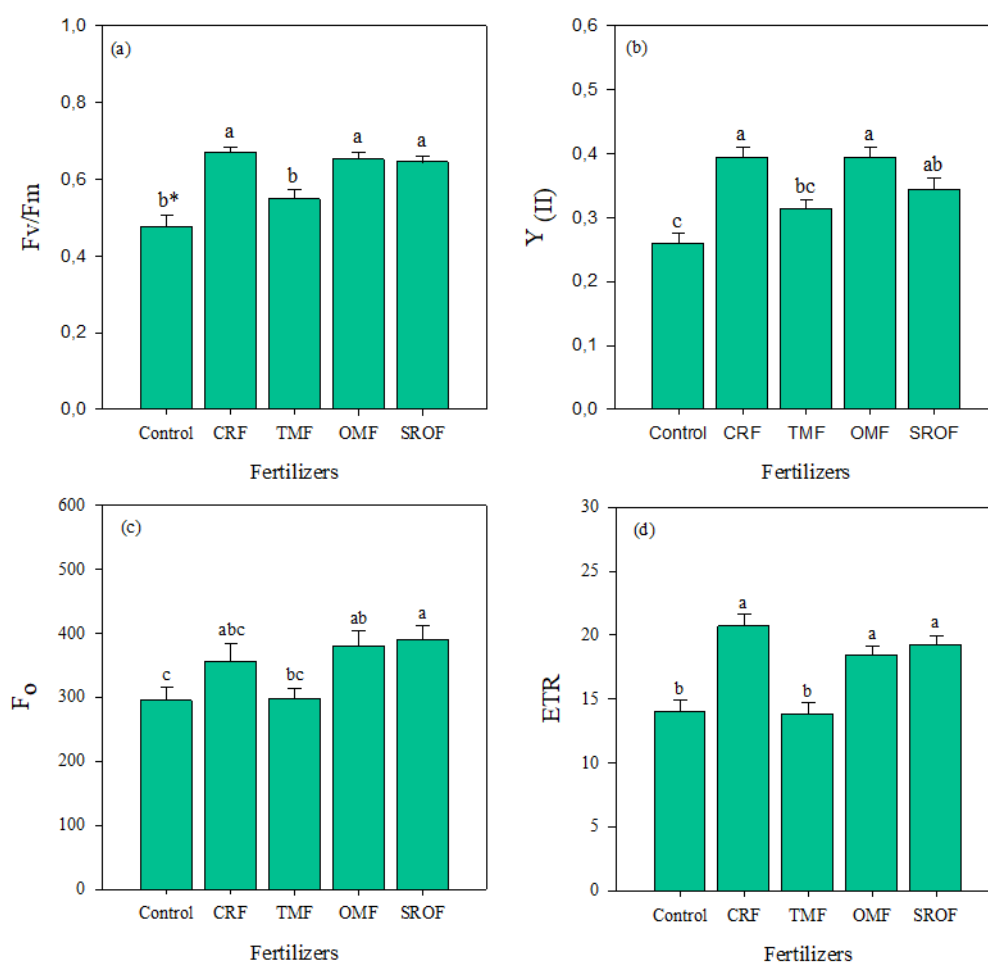


Figure 4. The effects of using different types of fertilizers on the maximum quantum yield of photosystem II (a) photochemical potential (b), initial fluorescence (c) and electron transport rate (d) of *Cordia americana* plants at 180 days after transplanting. Control (without basal fertilization), Controlled-release fertilizer (CRF), Traditional mineral fertilizer (TMF), Organic and mineral fertilizer (OMF), Soil-remineralizing organic fertilizer (SROF). \*Comparison of means using Tukey's test at a 5% probability of error. Vertical bars indicate mean  $\pm$  standard error values.

Figura 4. Efeito do uso de diferentes tipos de fertilizantes sobre o rendimento quântico máximo do fotossistema II (a) potencial fotoquímico (b), fluorescência inicial (c) e taxa de transporte de elétrons (d) de plantas de *C. americana* aos 180 dias após o transplante. Controle (sem adubação de base), Fertilizante de liberação controlada (FLC), Fertilizante mineral tradicional (FMT), Fertilizante organomineral (FOM), Fertilizante organomineral remineralizador do solo (FOMRS). \*Comparação de médias pelo teste Tukey a 5% de probabilidade de erro. Barras verticais indicam a média  $\pm$  erro padrão.

## DISCUSSION

Our findings in this study revealed that *Cordia americana* plants generally performed better when fertilized with controlled-release (CRF), organic and mineral (OMF), and soil-remineralizing organic mineral (SROF) fertilizers combined with the inoculation of *Trichoderma harzianum*. In this regard, several studies have reported the benefits of using inocula of different species of *Trichoderma* for enhancing the growth of forest seedlings under nursery conditions. For example, Amaral *et al.* (2017) reported the highest values for height and stem diameter in *Jacaranda micranta* Cham. seedlings inoculated with *Trichoderma asperelloides* Samuels. In further studies, Griebeler *et al.* (2021) evaluated the use of beneficial microorganisms as a strategy for maximizing the growth of trees under field conditions, and found that root system inoculation with *T. asperelloides* and *T. harzianum* was favorable with respect to promoting the initial development of *Cordia trichotoma* (Vell.) Arráb. ex Steud.

It is speculated that the beneficial effects of *Trichoderma* spp. could be associated with multiple factors, including an enhancement of the absorption potential and solubilization of nutrients within the rhizosphere

(SOLDAN *et al.*, 2018) and stimulation of plant cell multiplication via the production of indole-3-acetic acid (CHAGAS *et al.*, 2016). In practical terms, in addition to enhancing the growth and vigor of plants, the use of this type of biological amendment can reduce the need for fertilization in some crops (CHEN *et al.*, 2011), as well as the use of chemical products, thereby reducing the associated production costs and dependence on chemical inputs.

With regards to fertilizer usage, the positive effects of CRF on the growth of *C. americana* plants were evident based on our assessments of stem diameter, leaf area, and dry mass. For example, compared with the control treatment, we detected increases of 150% and 260% in the allocation of leaf dry mass and leaf area, respectively, in plants receiving the CRF. An increase in plant leaf area is associated with an increase in light interception, which is turn conducive to promoting increases in biomass productivity (MEZZOMO *et al.*, 2018). Thus, it can be inferred that the larger leaf area promoted by the application of CRF would contribute to higher growth rates in forest species. Similarly, compared with the control treatment, we recorded an increase of 280% in the allocation of root dry mass in plants receiving the CRF treatment. We can thus assume that partitioning of the nutrient supply over time contributed to a sustained adequate supply of nutrients to the *C. americana* plants, thereby resulting in greater root production, particularly that of fine roots, in plants grown using CRF.

Our results in this regard are consistent with those reported by Rossa *et al.* (2015), who observed a higher average dry mass allocation in *Eucalyptus grandis* (Hill, ex-Maiden) plants fertilized using CRF. The benefits obtained from using controlled-release fertilizers are associated with a maintenance of the synchrony between nutrient release and the growth and developmental needs of plants (GUO *et al.*, 2017), and also contributes to minimizing damage to plant root system caused by the accumulation of nutrients to toxic levels, as well as reducing leaching-related losses.

The use of organic and mineral fertilizers derived from swine waste has been proven to be a viable alternative for the cultivation *C. americana*, and in the present study, we found that compared with plants cultivated using TMF, plants treated with OMF and SROF were characterized by higher average dry mass allocation and leaf area. Similar results have been reported by Barros *et al.* (2022), who observed higher growth rates in *Eucalyptus grandis* plants cultivated with OMF and SROF. Consistently, Ros *et al.* (2018) found that organic compost derived from treated swine wastewater via mechanized composting was effective in producing seedlings of *Eucalyptus* spp., *Toona ciliata* var. *australis*, and *Khaya ivorensis* A. Chev.

The positive growth responses of *C. americana* treated with organic and mineral fertilizers derived from swine manure can be explained in terms of the properties of these waste products, with an increase in organic matter associated with the high content of nutrients, mainly N, being made available to plants (ARAÚJO *et al.*, 2019). According to Miyazawa and Barbosa (2015), given that swine manure contains multiple nutrients necessary for plant development, it can be used as fertilizer, and after being absorbed and metabolized, the nutrients are used to build biomolecules, thereby resulting in an increase in morphometric variables.

In this study, we also established that the use of different types of fertilizer can have a significant influence the Falker chlorophyll indices ICFa and ICFb, with CRF treated *C. americana* plants being characterized by superior responses compared with those observed in plants receiving the other assessed treatments. Similarly, Griebeler *et al.* (2023) observed higher chlorophyll indices in *Citharexylum montevidense* (Spreng.) Moldenke plants cultivated using a controlled-release fertilizer as a basal dressing. Chlorophyll indices are indirect indicators that enable inferences regarding the contents of photosynthetic pigments in plants. Chlorophyll content is an important physiological factor that is directly associated with plant photosynthesis. Higher levels of chlorophyll *a* and *b* result in greater energy transfer from the antenna complexes to the reaction centers of photosystems (TAIZ; ZEIGER, 2015), thereby indicating the potential for higher growth rates and biomass production.

Although the ICF can be used to assess the physiological status of forest species, there is as yet a lack of consensus regarding the indicative nature of the values obtained. Thus, considering the results of the present study, ICFa and ICFb values close to 30 and 12, respectively, can be assumed to be optimal for the species under study, given that the plants exhibited satisfactory performance.

Chlorophyll *a* fluorescence reflects the intensity of photochemical reactions in chloroplasts, as well as the physiological states of quantum light transmission, and can be used to estimate the degree of damage to the PSII reaction center (ZHANG *et al.*, 2018) and energy losses resulting from stress. In this study, the highest values of  $F_v/F_m$ ,  $Y_{(II)}$ ,  $F_o$ , and ETR were observed in plants cultivated with CRF, OMF, or SROF, and indicate that these plants had adequate physiological conditions for growth. The highest  $F_v/F_m$  values ( $\approx 0.70$ ) in *C. americana* were recorded in plants cultivated with the application of controlled-release or organic and mineral fertilizers, which can be considered indicative of a greater use of light energy channelled into biomass production.

Consistently, studies on native forest species have considered  $F_v/F_m$  values greater than 0.60 to be indicative of adequate initial plant growth (MEZZOMO *et al.*, 2018; GRIEBELER *et al.*, 2023). The  $F_v/F_m$  ratio is an attribute that expresses the relative efficiency of the absorption of light energy by the PSII antenna complex and its respective conversion to chemical energy, and accordingly, this ratio can serve as an important index for



identifying changes in the photosynthetic apparatus of plants that may be induced by stress associated with a lack of water or nutrients. In the present study, we found that this parameter enabled us to gain a reasonable assessment of the effects of applying different types of fertilizer on the maximum efficiency of PSII in *C. americana*, with higher values being consistent with more pronounced increases in growth and biomass allocation.

The parameter  $Y_{(II)}$  represents the effective quantum yield of PSII, with elevated values taken to be indicative of the conversion of a greater portion of the photons absorbed by PSII to chemical products (KALAJI *et al.*, 2014). Conversely, lower values are considered to reflect a reduction in the rate of electron transport and, consequently, in the capacity to assimilate CO<sub>2</sub> (XIE *et al.*, 2018). Similarly, the higher values of ETR recorded for plants fertilized with CRF, OMF, and SROF can be considered indicative of an elevated rate of electron transport associated with photochemical reactions compared with that in plants receiving the other fertilization treatments. Thus, by reflecting the effective functioning of the plant photosynthetic apparatus, and specifically an enhancement in the efficiency of light energy conversion and the CO<sub>2</sub> assimilative capacity in the PSII reaction center, the values obtained for  $Y_{(II)}$  and ETR serve to confirm the positive effects of using these fertilizers in the cultivation of *C. americana*. These findings thus enabled us to identify CRF, OMF, and SROF fertilizers as suitable nutrient inputs for the cultivation *C. americana*, as evidenced by increases in chlorophyll indices and a more efficient utilization of light energy in the photosynthetic processes, resulting in plants with higher rates of growth, dry mass allocation, and leaf area. Moreover, it is important to highlight the fact that the utilization of fertilizers derived from swine manure, and potentially other residues and waste products, in seedling production and forest stand establishment represents an important alternative to the disposal of such wastes, thereby making a valuable contribution to society and the environment.

## CONCLUSIONS

- Inoculation of *T. harzianum* in conjunction with the application of slow-release fertilizers or manure-based organic and mineral fertilizers is effective for promoting the initial growth of *C. americana* plants.
- The use of a controlled-release fertilizer has a positive influence on all evaluated plant attributes and is effective in maximizing the initial development of *C. americana* plants.
- The use of granulated fertilizers derived from swine manure (OMF and SROF) enhances plant growth and photosynthetic efficiency, thereby identifying these fertilizers as viable alternatives for the cultivation of young *C. americana* plants.

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