

# GROWTH AND PHYSIOLOGY OF *Balfourodendron riedelianum* SEEDLINGS IN THE NURSERY AND IN THE FIELD

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## Resumo

*Crescimento e fisiologia de Balfourodendron riedelianum no viveiro e no campo.* O estudo teve como objetivo verificar o efeito de diferentes volumes de recipiente e doses de fertilizante de liberação controlada (FLC) sob aspectos morfofisiológicos de mudas de *Balfourodendron riedelianum* na fase de viveiro e em campo. Para a produção das mudas foram utilizados três volumes recipientes com diferentes volumes (tubetes de polipropileno de 180 e 280 cm<sup>3</sup> e sacos plásticos de 500 cm<sup>3</sup>) e quatro doses de fertilizante de liberação controlada FLC (0, 4, 8 e 12 g L<sup>-1</sup> de substrato). Ao final da fase de produção de mudas, 240 dias após a semeadura, mensurou-se a altura da parte aérea (H), diâmetro do coleto (DC), massa seca aérea, radicular e total, área foliar e a fluorescência da clorofila *a* e *b*, calculada a relação H/DC e o Índice de Qualidade de Dickson. Os mesmos tratamentos foram avaliados em campo, 540 dias após o plantio, sendo mensurado a sobrevivência, o incremento em altura da parte aérea e diâmetro do coleto, massa seca aérea, área foliar, fluorescência da clorofila *a* e índice de clorofila *a*, *b* e total. Como resultados, a adubação de base com uso de FLC teve influência positiva na produção de *Balfourodendron riedelianum*. Assim, recomenda-se o uso de tubete de 180 cm<sup>3</sup> associado à dose de 12 g L<sup>-1</sup> de FLC, para a produção de mudas. Adicionalmente, os resultados obtidos no viveiro, para a produção de mudas, são confirmados no campo.

*Palavras-chave:* Mudas florestais; recipiente; nutrição mineral.

## Abstract

The present study aimed to determine the effect of different container volumes and doses of controlled release fertilizer (CRF) on the morphophysiological aspects of *Balfourodendron riedelianum* seedlings in the nursery and verify if these responses were replicated in the field. For the production of seedlings in nursery, three container volumes (180 and 280 cm<sup>3</sup> polypropylene tubes and 500 cm<sup>3</sup> plastic bags) and four doses of CRF (0, 4, 8, and 12 g L<sup>-1</sup> of substrate) were tested, and the seedlings were grown for 240 days. At the end of the nursery period, the following parameters were measured: height (H); stem diameter (SD); dry mass of shoot, root, and total; root length; leaf area; and chlorophyll fluorescence. The H/SD ratio and the Dickson Quality Index were calculated. The same treatments were evaluated in the field at 540 days after planting. Survival, height, and diameter increase, aerial dry mass, leaf area, chlorophyll *a* fluorescence and chlorophyll index (*a*, *b* and total) were measured. Basic fertilization using CRF had a positive influence on the production of *B. riedelianum* seedlings. It is recommended to use a 180 cm<sup>3</sup> tube and a dose of 12 g L<sup>-1</sup> CRF for the production of seedlings. The results obtained in the nursery for the production of seedlings were confirmed to occur in the field.

*Keywords:* Forest seedlings; container; mineral nutrition.

## INTRODUCTION

Brazil is considered to be among the countries with the greatest forest diversity in the world; however, deforestation has reduced native forest areas (FAO, 2011). Therefore, several initiatives have been aimed at restoration and conservation; however, this has increased the need for quality forest seedlings of native species (RODRIGUES *et al.*, 2009).

Among the many endangered native forest species that are in need of urgent conservation measures is *Balfourodendron riedelianum* (KUBOTA *et al.*, 2015). This species belongs to the family Rutaceae (LORENZI, 2002), occurring naturally in Argentina, Paraguay, and Brazil, where it exhibits the behavior of a semideciduous and heliophyte species, and is classified from pioneer to late secondary (CARVALHO, 2003).

*B. riedelianum* is noted for its ecological and economic importance and is suitable for the restoration of degraded areas and commercial reforestation; in addition, the wood can be used for the manufacture of luxury furniture (CARVALHO, 2003). The species has the potential for the elaboration of agrochemicals, due to the presence of secondary metabolites that are capable of inhibiting the photosynthetic process (VEIGA *et al.*, 2013).

The production of seedlings of some native species remains a challenge; therefore, it is necessary to establish strategies and appropriate protocols (DUTRA *et al.*, 2016). In forest nurseries, the main factors that affect seedling quality are seed quality, container type, substrate, fertilizer, and irrigation (BERGHETTI *et al.*, 2019; MEZZOMO *et al.*, 2018). The container interferes with the development of the root system and may cause restrictions during the period in the nursery; moreover, its characteristics and dimensions may influence operational aspects (LUNA *et al.*, 2009). Tubes and plastic bags are the containers most commonly used for the production of native forest seedlings.

Another important aspect in seedling production is basic fertilization, which is fundamental to maximize the growth of seedlings in containers (KLOOSTER *et al.*, 2012) and must be carefully analyzed, as both excess and lack of nutrients can have a negative influence on growth (GONÇALVES *et al.*, 2005). The efficiency of nutrient use is related to the dose and source of fertilizers used (BERGHETTI *et al.*, 2019). The installment of the applications an important way to maximize efficiency, but this practice increases operating costs. The use of a controlled-release fertilizer (CRF) is an option because it provides nutrients in a regular and ongoing basis to the seedlings (ELLI *et al.*, 2013), which prevents damage to the root system due to toxic concentrations and/or changes in the osmotic potential of the substrate, and reduces the losses by lixiviation.

In addition, the determination of methodologies and inputs for nursery production should be confirmed in the field to recommend techniques that heavily favor the growth of seedlings (GASPARIN *et al.*, 2014), thereby avoiding the wrong conclusions (VALLONE *et al.*, 2009). Thus, the present study aimed to investigate the effect of using different volumes, types of containers, and rates of controlled-release fertilizer by the assessment of morphophysiological aspects *B. riedelianum* seedlings in the nursery, and verify that the responses obtained in the nursery also occur in the field.

## MATERIAL AND METHODS

### Nursery Experiment

The experiment was conducted at the Laboratory of Forestry and Forest Nursery (29° 43' S and 53° 43' W), Federal University of Santa Maria (UFSM), in Santa Maria, RS, from September 2013 to September 2014. In accordance with the Köppen classification, the climate of the region is subtropical, Cfa type, with an annual average precipitation of 1720 mm and an annual average temperature of 19.1°C.

The fruits of *B. riedelianum* were collected in August 2013 from trees located in forest fragments in the municipality of Nova Palma, RS. After collection, the fruits were placed in trays in a covered and airy place for pre-drying for 3 days at room temperature. Afterwards, processing was performed by using pruning shears, removing the winged part of the fruit. After processing, the fruits were stored in a cold and humid chamber (T° = 8°C, U.R. = 80%) until planting.

The planted fruit was placed in plastic trays with sifted sand (2 mm mesh) and assigned to a greenhouse for 4 months for seedling emergence with irrigation of 8 mm day<sup>-1</sup>. In January 2014, the seedlings with a height between 5 and 7 cm and a pair of leaves were transplanted into the final container.

The experimental design was completely randomized, with three replicates; a 3 × 4 factorial scheme, comprising three container volumes (180 and 280 cm<sup>3</sup> polypropylene tubes and 500 cm<sup>3</sup> plastic bags) and four controlled-release fertilizer doses: (0 (control), 4, 8, and 12 g L<sup>-1</sup> substrate). The experimental plot consisted of 20 seedlings.

The substrate used consisted of sphagnum peat and charred rice hulls in a 2:1 ratio. The substrate preparation was conducted in a concrete mixer and was performed after the containers were filled and the substrate was accommodated. The CRF used was Osmocote®, which has the following chemical composition: 15% nitrogen; 9% superphosphate; 12% potassium chloride; 1% magnesium, 2.3% sulfur; 0.05% copper; 0.06% manganese; 0.45% iron; and 0.2% molybdenum, and is coated with a semipermeable organic layer that allows nutrient release for between 5 and 6 months.

At 240 days after transplanting, the shoot height (H) of plants was measured by using a millimeter ruler and the stem diameter (SD) was measured by using a digital caliper (accuracy 0.01 mm). From these data, the H/SD ratio was obtained. Leaf area (LA) quantification was performed on two seedlings per repetition. The shoot was separated into the stem and leaves; the parts were distributed on white paper and covered by a transparent glass with a scale reference, photographed by using a digital camera, and processed by using the ImageJ® program.

The shoot dry weight (SDW) and root dry weight (RDW) were determined by using the same samples LA. The aerial part and the root part were packed in paper (Kraft type) packages and placed in a forced air oven to dry at 65°C±5°C for 72 h. The samples were weighed on analytical balance (precision 0.01 g) until a constant weight was reached. The total dry weight (TDW) was obtained from the sum of SDW and RDW. From these data, the Dickson Quality Index (DQI) was calculated.

The analysis of chlorophyll *a* fluorescence was performed by using pulse fluorometry; a modulated Junior-PAM Chlorophyll Fluorometer (Walz Mess-und-Regeltechnik) determine the change in each replicate through the analysis of a fully expanded leaf from the second branch, which was pre-adapted to the dark for 30 minutes in aluminum foil. After this period, the analysis was performed for between 7 and 11 hours, to obtain the values of initial fluorescence (F0), maximum fluorescence (Fm), maximum quantum yield (Fv/Fm), and electron transport rate (ETR).

## Field experiments

The seedlings were planted in October 2014 in an experimental area belonging to UFSM, in Santa Maria (29° 43' 12"S and 53° 43' 14" W). The same treatments described in the nursery experiments were used. Prior to planting, soil fertility was characterized by using chemical analysis. The results were then interpreted and pH corrected by liming 30 days before planting.

Initially, the area was completely cleaned by using a brushcutter and controlled by leaf-cutting ants. Subsequently, holes 30 cm in diameter and 35 cm deep were drilled by using a soil drill attached to a tractor, with a spacing of 1 m × 1 m. The seedlings were planted and then irrigated with 2 L of water per seedling. Fertilization was performed at three time points (30, 210, and 390 days after planting) by the application of 100 g NPK (05-20-20), in two lateral wells.

The experiment was used a randomized block design, where the 12 treatments evaluated in the nursery were tested: type of container and doses of controlled-release fertilizer. The experimental design consisted of three blocks; each plot consisted of four seedlings with an average treatment size, totaling 144 plants.

Height (H) and stem diameter (SD) evaluations were performed at the time of planting and at the end of the experiment (0 and 540 days after planting). From the final measurement, performed at 540 days after planting, the increase in height (IH) and the increase in diameter of the seedlings (ISD) of the seedlings in relation to the time of planting were calculated. Survival assessment was performed at 540 days after planting.

At 540 days after planting, the chlorophyll *a* fluorescence was evaluated in one seedling from each block of treatment, by using the methodology of the experiments conducted in the nursery. Chlorophyll *a* and *b*, and total index were analyzed in two plants per replicate, and the readings were taken in a completely expanded leaf of the third branch, by using a portable chlorophyll meter ClorofiLOG (Falker Automação Agrícola, Brazil), which provides a unit measurement of the Falker chlorophyll index (ICF).

## Statistical analysis

Data were subjected to analysis of residual normality and variance homogeneity assumptions by the Shapiro-Wilk and Bartlett test, respectively. Subsequently, the analysis of variance was performed, and the data were analyzed according to the model:  $Y_{ijk} = m + b_j + a_i + dk + ad_{ik} + \delta_{ijk}$ , where:  $Y_{ijk}$  was the observed value for each experimental unit;  $m$  was a constant;  $b_j$  was the effect of blocks;  $a_i$  was the fixed effect of CRF doses;  $dk$  was the fixed effect of the container volume;  $ad_{ik}$  was the effect of the interaction between CRF doses and container volume; and  $\delta_{ijk}$  was the effect of the random error incident between experimental units. When a difference between treatments was found, polynomial regression analysis ( $p < 0.05$ ) was performed using the Sisvar statistical package. (FERREIRA, 2014).

## RESULTS

### Nursery Experiments

In the morphological variables of *B. riedelianum* seedlings observed at 240 days after subculturing, there was a significant interaction between container volumes and CRF doses only for leaf area. The other variables, height, stem diameter, height/stem diameter, shoot dry weight, root dry weight, total dry weight, Dickson quality index, and root length, were significantly different depending on the dose of CRF.

The development in height, stem diameter, and height/diameter showed a linear increase as a function of increasing CRF dose (Figure 1). The highest growth was observed at 12 g L<sup>-1</sup> CRF, at 12.32 cm, representing an increase of 88.96% compared with the control (0 g L<sup>-1</sup>). For the stem diameter and height/stem diameter, the largest averages of 2.58 and 4.9 mm, respectively, were also observed at the 12 g L<sup>-1</sup> CRF, an increase of 41.76% and 34.99%, respectively, compared with the control treatment.

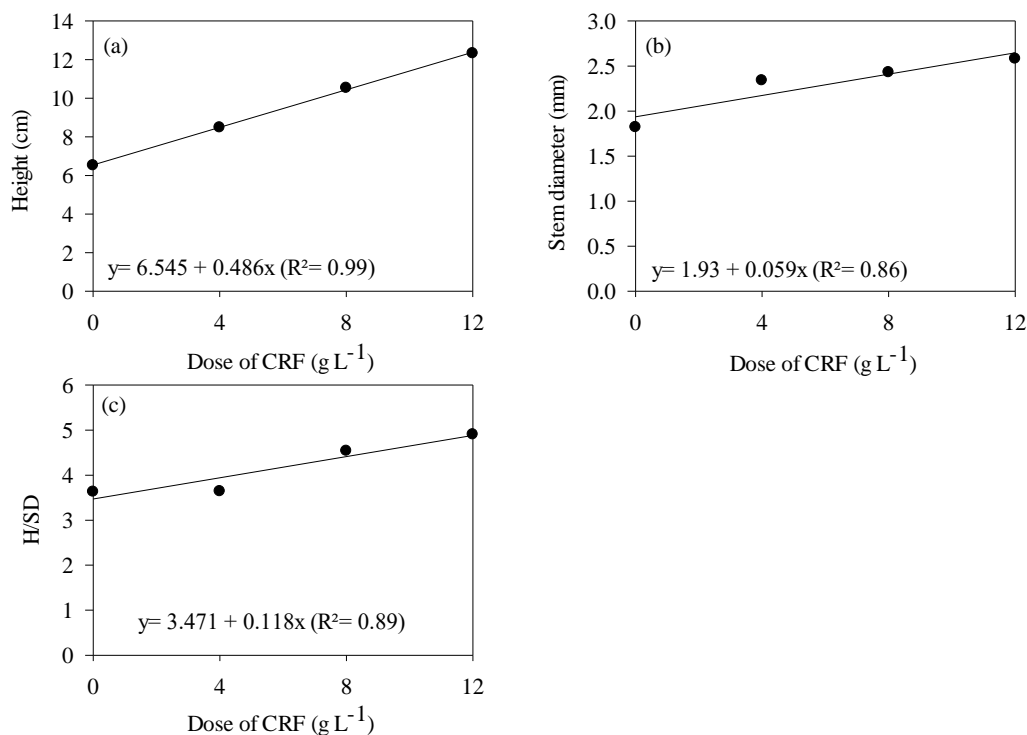


Figura 1. Altura (a), diâmetro do coleto (b) e relação altura/diâmetro do coleto (c) de mudas de *Balfourodendron riedelianum*, em função de doses de fertilizante de liberação controlada (FLC), aos 240 dias após repicagem.

Figure 1. Height (a), stem diameter (b) and height/stem diameter (c) of *Balfourodendron riedelianum* seedlings, as a function of doses of controlled release fertilizer (CRF), 240 days after transplanting.

The values of SDW, TDW, and DQI also showed a linear and increasing trend in growth as CRF dose increased; the highest averages (0.38 g seedling<sup>-1</sup>, 0.52 g seedling<sup>-1</sup>, and 0.09 seedling<sup>-1</sup>, respectively) were obtained for the 12 g L<sup>-1</sup> dose (Figure 2d). RDW and root system length exhibited quadratic behavior as the CRF dose increased (Figure 2b), with the highest averages also observed for the 12 g L<sup>-1</sup> dose (0.14 g seedling<sup>-1</sup> and 352.21 cm, respectively).

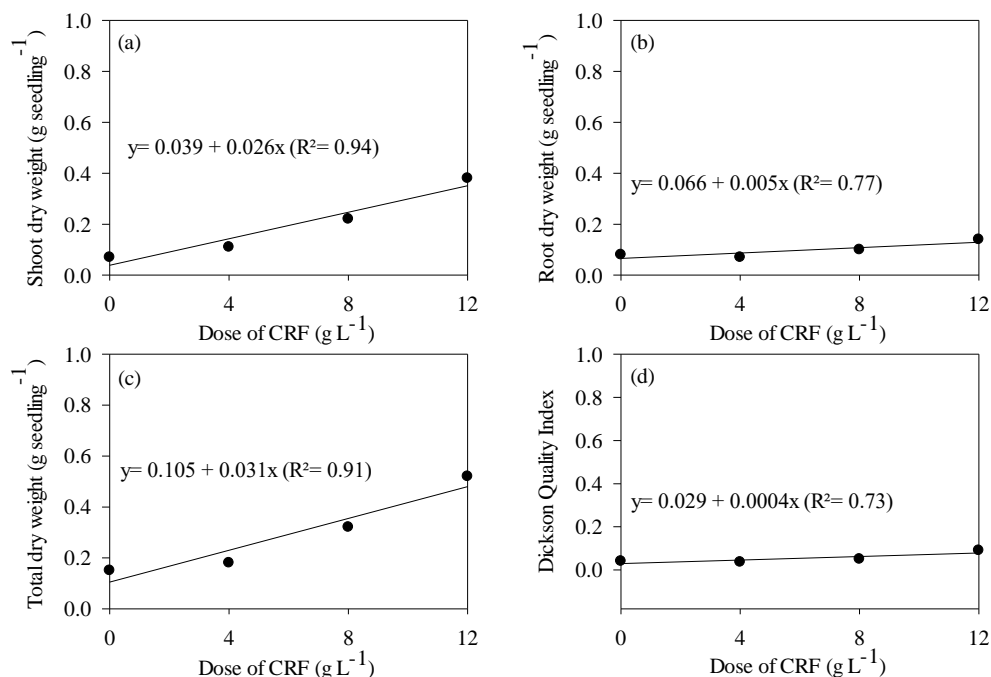


Figura 2. Massa seca aérea (a), massa seca radicular (b), massa seca total (c) e índice de qualidade de Dickson (d) de mudas de *Balfourodendron riedelianum*, em função das doses de fertilizante de liberação controlada (FLC), aos 240 dias após repicagem.

Figure 2. Shoot Dry weight (a), root dry weight (b), total dry weight (c) and Dickson quality index (d) of *Balfourodendron riedelianum* seedlings, according to the doses of controlled release fertilizer (CRF), 240 days after transplanting.

The leaf area followed an increasing trend as the CRF dose was increased for all container volumes, with the highest averages found at the 12 g L<sup>-1</sup> CRF dose (Figure 3). The seedlings grown in the 500 cm<sup>3</sup> container had a leaf area of 132.23 cm<sup>2</sup>, which was higher than those observed for the 180 and 280 cm<sup>3</sup> seedlings (93.08 and 80.42 cm<sup>2</sup> seedlings<sup>-1</sup>, respectively).

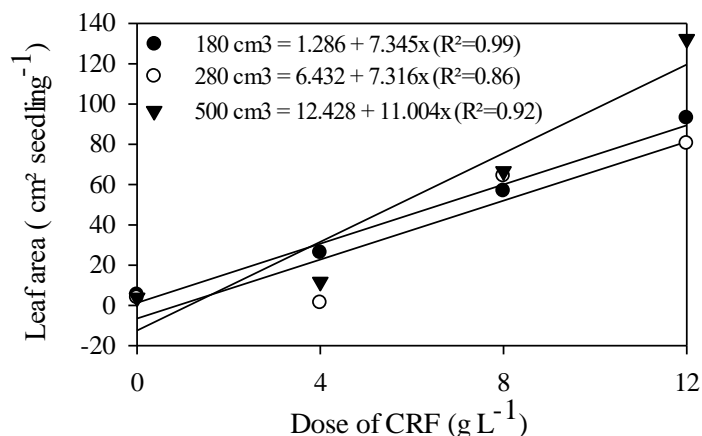


Figura 3. Área foliar de mudas de *Balfourodendron riedelianum*, produzidas em diferentes volumes de recipiente, em função das doses de fertilizante de liberação controlada (FLC), avaliadas aos 240 dias após repicagem.

Figure 3. Leaf area seedlings *Balfourodendron riedelianum* produced in different volumes of the container, depending on the controlled release fertilizer doses (CRF), evaluated 240 days after transplanting.

The physiological variables F0, Fm, Fv/Fm, and ETR showed no significant difference for each of the CRF doses and container volumes. A significant effect was found on maximum fluorescence (Fm) and maximum quantum yield (Fv/Fm) only for the CRF dose factor (Figure 4), with the highest averages observed at the 12 g L<sup>-1</sup> dose 526 and 0.61, respectively.

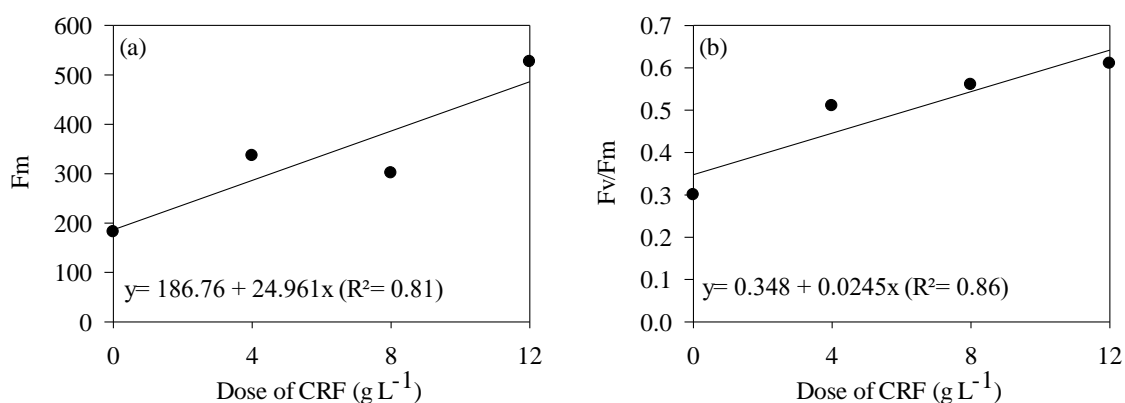


Figura 4. Fluorescência máxima (a) e do rendimento quântico máximo (b) de mudas de *Balfourodendron riedelianum*, em função das doses de fertilizante de liberação controlada (FLC), avaliadas aos 240 dias após repicagem.

Figure 4. Maximum fluorescence (a) and maximum quantum yield (b) of *Balfourodendron riedelianum* seedlings, as a function of controlled release fertilizer (CRF) doses, evaluated at 240 days after subculture.

## Field experimente

At 540 days after planting there was no significant interaction between CRF doses and container volumes for all morphological variables analyzed. Significant effect was verified only for the factor CRF doses, with increasing linear behavior.

At the end of the experiment the highest survival rate in the field was 64% for seedlings produced with 12 g L<sup>-1</sup> CRF, whereas the control treatment (0 g L<sup>-1</sup>) had the lowest survival rate (11%) (Figure 5).

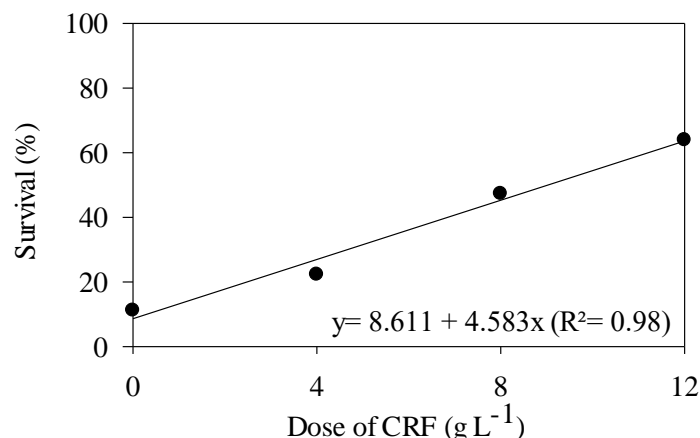


Figura 5. Sobrevivência (%) de mudas de *Balfourodendron riedelianum*, em função das doses de fertilizante de liberação controlada (FLC), aos 540 dias após plantio no campo.

Figure 5. Survival (%) of *Balfourodendron riedelianum* seedlings, depending on the doses of controlled release fertilizer (CRF), at 540 days after planting in the field.

Due to the high mortality observed in the control treatment seedlings (89%) it was not possible to include it in the statistical analysis of the morphological and physiological variables data performed at 540 days after planting. The increase in height, increase in stem diameter, aerial dry mass and leaf area of *B. riedelianum* seedlings showed linear behavior in response to increasing CRF dose (Figure 6), with the highest averages observed for the 12 g L<sup>-1</sup> CRF dose; this trend was the same as that observed in the nursery experiments.

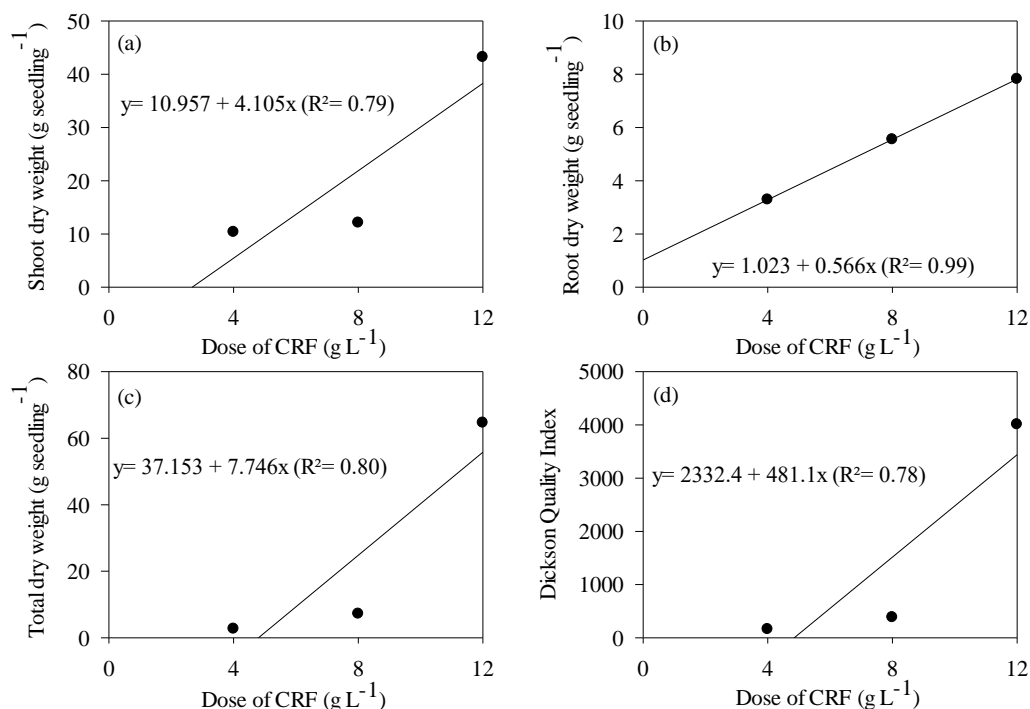


Figura 6. Incremento em altura (a), incremento em diâmetro do coleto (b), massa seca aérea (c) e área foliar (d) de mudas de *Balfourodendron riedelianum*, em função das doses de fertilizante de liberação controlada (FLC), avaliadas aos 540 dias após plantio no campo

Figure 6. Increase in height (a), increase in shoot diameter (b), shoot dry mass (c) and leaf area (d) of *Balfourodendron riedelianum* seedlings, according to the doses of controlled release fertilizer (FLC) evaluated at 540 days after planting in the field.

The analysis of physiological variables of *B. riedelianum* seedlings in the field at 540 days after planting revealed no significant interaction between CRF dose and container volume. A significant effect on the parameter of maximum quantum yield ( $F_v/F_m$ ) was observed only for CRF dose, which followed an increasing linear trend with an increase in CRF dose (Figure 7).

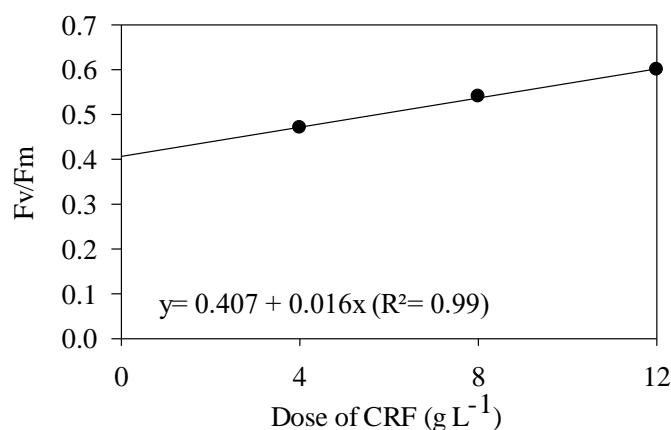


Figura 7. Rendimento quântico máximo ( $F_v/F_m$ ) de mudas de *Balfourodendron riedelianum*, em função das doses de fertilizante de liberação controlada (FLC), na fase de viveiro, avaliadas aos 540 dias após plantio no campo.

Figure 7. Maximum quantum yield ( $F_v/F_m$ ) of *Balfourodendron riedelianum* seedlings, as a function of the doses of controlled release fertilizer (CRF), at the nursery stage, evaluated at 540 days after planting in the field.

## DISCUSSION

The largest growth in height and stem diameter and the highest height/stem diameter ratio were observed when using 12 g L<sup>-1</sup> CRF, which demonstrated the marked influence of CRF dose on seedling growth. This may have been because higher doses of fertilizer resulted in greater contact of the roots with the nutrients, allowing the greater absorption and accumulation of nutrients in the organs of plants; thus, there is an increase in the metabolic activity of plants, increasing their growth. Klooster *et al.* (2012) studied the growth and physiology of shadow species in response to CRF use, and found that height tended to increase with the addition of fertilizer. These results suggested that CRF improved the initial establishment of seedlings in the field; similarly, Sloan and Jacobs (2012) reported that the use of CRF increased the operational efficiency and potential of photosystem II (PSII), which contributed to an increase in net photosynthetic rate, thereby providing better seedling development.

For *B. riedelianum*, only the results of the height/diameter ratio of the collection are considered adequate, as recommended by Gonçalves *et al.* (2005), who consider a quality seedling to have a height between 20 and 35 cm, a diameter between 3 and 10 mm and a height/stem diameter ratio between 2 and 7. However, considering the slow growth of the species in the nursery (CARVALHO, 2003), values below these are acceptable, as observed in this study.

The lower accumulation of SDW and RDW in seedlings produced with 0 g L<sup>-1</sup> CRF was due to the lower nutritional availability, making the roots a strong carbohydrate drain, thus causing greater limitation to shoot growth than of the root. Similar results were observed by Rossa *et al.* (2014), who studied different doses of CRF in the production of *Schinus terebinthifolius* seedlings, observed that the lowest dose of CRF (0 g L<sup>-1</sup>) produced seedlings with the lowest mean SDW and RDW and the balance between the values of these variables. In addition, the increase in CRF dose allowed a greater increase of SDW in relation to RDW, with the highest averages found in the highest dose used (10 g L<sup>-1</sup>). In this sense, Batista *et al.*, (2014) considered that the higher the value of this variable, the better the quality of the seedling.

The length of the root system increased by 121.03% (192.86 cm) when 12 g L<sup>-1</sup> was used compared with seedlings produced without the use of CRF. The same trend was observed in the RDW, with an increase of 75% (0.06 g) compared with the control, but this value was smaller than the percentage increase observed for root length. This difference may be due to the higher number amount of thin roots observed in seedlings produced with 12 g L<sup>-1</sup> CRF. According to Haase (2008), root mass does not always dictate root fibrosity, as a seedling with a large amount of thin roots may have the same mass as a more developed pivoting root seedling.

The responses of forest species seedlings to fertilizers has been widely interpreted in terms of leaf area increase. According to Mezzomo *et al.* (2018), the increase in leaf area index is associated with the increase in light interception, which serves as the main promoter an increase in biomass productivity. In addition, the larger leaf area from fertilization also results in increased growth efficiency. Thus, it can be inferred that the growth response of seedlings submitted to different levels of fertilization is driven by changes in plant leaf area.

Maximum fluorescence exhibited a linear increase as CRF dose increased. The lowest average was observed for plants produced without the use of CRF (0 g L<sup>-1</sup>), with a value of 181.94. The lower Fm values may be related to the photoreduction of quinone A (QA), associated with PSII inactivation in the thylakoid membranes, compromising the electron flow between the photosystems (SILVA *et al.*, 2006).

The highest value of the maximum quantum yield (Fv/Fm) (0.61), obtained in seedlings produced with 12 g L<sup>-1</sup> CRF. Turchetto *et al.* (2016), who studied the performance of different typical species of undergrowth in nursery, proposed that intermediate values (above 0.55) were considered good predictors of seedling survival and early growth.

The increase in Fv/Fm with the increase in FLC doses indicated that fertilization at transplantation increased PSII operational efficiency. Such increases in PSII operational potential and potential probably contributed to the increase in net photosynthetic rate.

For morphological and physiological variables, the highest CRF dose (12 g L<sup>-1</sup>) provided the best seedling performance. However, the results also indicated that there was further scope to increase seedling quality. This suggested that the species may need w higher dose of CRF to achieve its maximum growth, supporting the need for further studies on the subject. The volume of the container influenced only the leaf area, with the largest container and the largest substrate volume (500 cm<sup>3</sup>) permitted the greatest growth.

In the field, the higher survival rates of the seedlings produced at the highest doses of CRF were associated with the quality of the seedlings at the time of planting, as seedlings with better morphological and physiological characteristics in the nursery exhibited longer survival. Gasparin *et al.* (2015) evaluated the field performance of *Parapiptadenia rigida* seedlings produced with different doses of CRF, and observed that the lowest survival rate occurred for seedlings that did not receive FLC fertilization (0 g L<sup>-1</sup>).

In addition, the best results for the morphological variables in the field were observed for seedlings produced in the nursery with a dose of 12 g L<sup>-1</sup> CRF; this may be related to the higher root dry mass and longer root length found in the nursery samples that received this treatment.

According to Haase (2008), the more developed the root system, the greater the survival and growth of the seedlings, with a positive correlation between root dry mass and shoot height.

Thus, seedlings produced with 12 g L<sup>-1</sup> CRF showed the best results for all variables analyzed in the field at 540 days after planting; however, different container volumes did not influence the establishment and growth of *B. riedelianum* seedlings, confirming the results obtained in nursery. In addition, it is noteworthy that *B. riedelianum* is a demanding species and responsive to high doses of fertilizer; therefore, studies that define its nutritional demand are required to maximize its production.

## CONCLUSIONS

- Basic fertilization using controlled-release fertilizer (CRF) had a positive influence on the production of *Balfourodendron riedelianum* seedlings.
- It is recommended to use a 180 cm<sup>3</sup> tube and 12 g L<sup>-1</sup> dose of CRF to produce seedlings of this species.
- Results obtained in the nursery for the production of *Balfourodendron riedelianum* seedlings were applicable to experiments conducted in the field.

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