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Nutrient management affects growth and quality of *Berberis laurina* seedlings, a medicinal species from South America

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ABSTRACT – In this study, we aimed to evaluate growth and quality of *Berberis laurina* seedlings submitted to increasing doses of controlled-release fertilizer (CRF). Each treatment was elaborated with different doses of CRF Basacote® Plus 9M: 0.0, 2.5, 5.0, 7.5, 10.0, and 12.5 kg m^{-3}. Seedlings were measured at 15, 30, 60, 90, and 150 days in height and stem diameter. At 150 days, we evaluated aerial dry biomass, root dry biomass, height/diameter ratio, and Dickson quality index. Plants without CRF did not show significant growth in height even after 150 days and treatments with 7.5, 10.0, and 12.5 kg m^{-3} CRF provided the best results. At dose 12.5 kg m^{-3}, there was a tendency to decrease height and diameter curves, indicating toxic amounts of FLC to plants growth in nursery. Considering better use of resources, we recommend 7.5 kg m^{-3} of CRF for the production of *B. laurina* seedlings.

Keywords: controlled-release fertilizer, espinho-de-são-joão, forest nursery, plant nutrition, silviculture

RESUMO – **Manejo nutricional no crescimento e qualidade de mudas de *Berberis laurina*, espécie medicinal da América do Sul.** Neste estudo, objetivamos avaliar o crescimento e qualidade de mudas de *Berberis laurina* submetidas a doses crescentes de fertilizante de liberação controlada (FLC). Cada tratamento foi elaborado com as respectivas doses de FLC Basacote® Plus 9M: 0,0, 2,5, 5,0, 7,5, 10,0 e 12,5 kg m^{-3}. As mudas foram avaliadas aos 15, 30, 60, 90 e 150 dias em altura e o diâmetro do colo e, aos 150 dias avaliamos biomassa seca aérea, biomassa seca radicular, relação altura/diâmetro e índice de qualidade de Dikson. As plantas do tratamento sem adição de fertilizante não apresentaram crescimento significativo em altura mesmo após 150 dias e os tratamentos com aplicação de 7,5, 10,0 e 12,5 kg m^{-3} de FLC proporcionaram os melhores resultados. Na dose 12,5 kg m^{-3} observou-se tendência de decréscimo das curvas de altura e diâmetro, indício de quantidades prejudiciais de FLC ao crescimento das plantas em viveiro. Ao considerar melhor aproveitamento do insumo, recomenda-se o uso de 7,5 kg m^{-3} para produção de mudas de *B. laurina*.

Palavras-chave: espinho-de-são-joão, fertilizante de liberação controlada, nutrição de plantas, viveiro florestal

INTRODUCTION

Berberis laurina Billb. (Berberidaceae) is a native species to Brazil, Uruguay, and Argentina; it occurs in the Mixed Ombrophilous Forest and Semideciduous Seasonal Forest (Pedralli 2002, Stehmann 2015). It is popularly known as espinho-de-são-joão, são-joão, and espinho-de-judeu. Other species of the genus *Berberis*, occurring mainly in Asia, are of great importance to the pharmaceutical industry due to their antiparasitic (Batiha *et al.* 2020), anticancer (Belwal *et al.* 2020) and antidiabetic action, among other metabolic disorders (Gu *et al.* 2020). Despite being a species with a medicinal appeal (Martins-Ramos *et al.* 2010), studies related to the development of products from *B. laurina* run into the absence of silvicultural information (Althaus *et al.* 2005).

Restoration of degraded areas where the species occurs has been increasingly intense, requiring large amounts of most varied species seedlings and increasing the interest in species with potential for commercial use. Seedling quality is among the fundamental aspects of the success of plantations; this quality is usually determined from plants' morphological and physiological characteristics (Davis & Jacobs 2005, Ivetić *et al.* 2016, Grossnickle & MacDonald 2018, Riikonen & Luoranen 2018). Despite the existence of pre-established standards for seedling quality, especially species used in commercial plantations, it is clear that the intrinsic characteristics of each species should be considered for the establishment of appropriate standards for each situation (Dumroese *et al.* 2016, Khanal *et al.* 2018).

The appropriate use of fertilizers is among the most important factor for quality seedlings production and reduction of environmental impacts in the forest industry (Cortina *et al.* 2013). Both the sources and doses of fertilizers to be applied should be considered to optimize resource use (Madrid-Aispuro *et al.* 2020). Several studies highlight the use of controlled-release fertilizers in forest seedlings production, ensuring high efficiency in nutrients availability with reduction of leaching losses or excess in fertilizer application (Silva *et al.* 2014, Stüpp *et al.* 2015, Brito *et al.* 2018, Cabreira *et al.* 2019, Madrid-Aispuro *et al.* 2020). Regarding the genus *Berberis*, field studies indicate changes in the nutritional composition of leaves according to fertilization (Arena *et al.* 2020).

We considered the hypothesis that controlled-release fertilizer doses would provide different growth and quality seedlings. Therefore, to determine the best nutrient management for *Berberis laurina* seedlings production, we evaluated seedling growth and morphological characteristics under increasing doses of controlled-release fertilizer.

MATERIAL AND METHODS

Seedlings of *B. laurina* were donated by the Chauá Society® and produced from seeds collected from mother trees in areas of species natural occurrence. The experiment was conducted from November/2019 to April/2020 in the forest nursery of the Forestry Sciences Department of the Federal University of Paraná (UFPR), Curitiba – PR, Brazil. The region climate is Cfb type, according to Köppen classification, characterized as humid subtropical with mild summer (Alvares *et al.* 2013).

Seedlings were produced in 110 cm³ polypropylene tubes filled with a commercial substrate composed of decomposed Pinus bark, carbonized Pinus bark, coconut fiber, and vermiculite (Tab. 1). The treatments consisted 0.0, 2.5, 5.0, 7.5, 10.0 and 12.5 kg m^{-3} doses of controlled-release fertilizer (CRF) Basacote® Plus 9M (16-8-12). After prick out, plants were maintained in a greenhouse, with four daily irrigations of 10-20 minutes via micro-sprinkler (114 L $hour^{-1}$), where they remained for 150 days.

To monitor plant growth, we evaluated seedlings height at 15, 30, 60, 90, and 150 days using a ruler (0.1 cm) and stem diameter at 30, 60, 90, and 150 days using a digital caliper (0.01 mm). At 150 days of production, we also evaluated shoot (SDB) and root (RDB) dry biomass; for this, we separated shoot and root of 5 plants of each repetition, the root part was washed in running water to remove substrate. We packed both parts in properly identified paper bags, which remained in a drying oven until constant weight (which happened after 72 hours) and, after this time, all plants parts were weighted on a 0.01 g precision scale. With these data, we calculated total dry biomass (TDB), height/diameter ratio (H/D), and Dickson Quality Index (DQI) (equation 1).

$$DQI = \frac{TDB}{\left(\frac{H}{D}\right)+\left(\frac{SDB}{RDB}\right)} \qquad (1)$$

where:
H = Height
D = Stem diameter
SDB = Shoot dry biomass
RDB = Root dry biomass
TDB = Total dry biomass

We set the experiment in a completely randomized design, with 6 treatments and 5 repetitions of 12 plants. Data normality was checked by the Shapiro-Wilk test ($p < 0.05$), variances homogeneity was checked by Bartlett test ($p < 0.05$), and then we subjected data to variance analysis in a split-plot design. Averages were compared with Tukey test ($p < 0.05$). Height, diameter, and dry biomass data were submitted to regression analysis to adjust models. We performed statistical analyses in the R software (R Core Team 2019).

RESULTS

Fertilizer doses influenced plant diameter and height from 30 and 60 days, respectively (Tab. 2). Plants without fertilizer application (0.0 kg m^{-3}) did not exhibit significant growth in height even after 150 days, indicating the importance of this input in forest seedlings production, regardless of the treatment. After this period, only plants with 7.5, 10.0 and 12.5 kg m^{-3} of CRF presented significant growth.

As for the variables mentioned above, dry biomass presented significant differences between CRF doses at 150 days (Tab. 3). We observed the highest values in plants with a higher concentration of fertilizer in the substrate (12.5 kg m^{-3}), indicating that CRF doses used in this experiment did not reach the toxicity dose for the species. Values of H/D ratio and DQI also indicated a progressive increase as fertilizer doses increased.

Although there is no significant difference between doses 10.0 and 12.5 kg m^{-3} of CRF for height and diameter at 150 days, it is possible to observe, from the regression adjustment equation (Fig. 1), that treatments approached the maximum dose for the species. These results indicate that doses much higher than those used in this experiment may be harmful to plant growth in nursery, although futures studies must confirm what is the maximum CRF dose for this species.

The adjustment of regression equations for the shoot, root, and total dry biomass indicated a gradual increase in this variable according to an increase in fertilizer doses (Fig. 2). However, the highest fertilizer dose (12.5 kg m^{-3}) resulted in a decrease in height and diameter. This difference indicates the importance of all growth variables and those consequences in the field when deciding CRF dose for a species.

Illustrating seedling performance at 150 days, Figure 3 presents root and shoot system of the most representative seedlings from each treatment. With this, we can observe the increase of seedlings biomass, corroborating the evaluations of quality and growth index.

Table 1. Bulk density (D), macroporosity (Macro), water holding capacity (WHC), total porosity (TP), electrical conductivity (EC), hydrogenionic potential (pH), and soluble salt content (SSC) of the substrate used for the production of *Berberis laurina s*eedlings.

Physical characteristics				Chemical characteristics		
D ($Kg\ m^{-3}$)	Macro (%)	WHC (%)	TP (%)	EC ($dS\ cm^{-1}$)	pH	SSC ($g\ L^{-1}$)
459.13	31.54	41.76	73.30	0.20	6.7	5.17

Table 2. Height (cm) and diameter (mm) of *Berberis laurina* seedlings submitted to increasing controlled-release fertilizer [Basacote® Plus 9M (16-8-12)] doses at 15, 30, 60, 90, and 150 days.

Doses	Days				
(Kg m-3)	15	30	60	90	150
			Height (cm)		
0.0	2.9 aA	2.9 aA	3.5 cA	2.8 cA	2.8 eA
2.5	2.9 aC	2.9 aC	3.5 cBC	5.1 cB	10.4 dA
5.0	3.7 aC	3.5 aC	4.8 bcC	8.2 bB	14.9 cA
7.5	3.1 aC	2.9 aC	3.9 cC	8.9 bB	20.9 bA
10.0	3.5 aD	3.4 aD	7.7 aC	15.3 aB	24.3 aA
12.5	3.6 aD	3.6 aD	7.2 abC	15.1 aB	24.2 aA
			Diameter (mm)		
0.0		0.94 bC	0.96 cBC	1.15 dAB	1.21 eA
2.5		1.03 abC	1.06 bcC	1.59 cB	2.21 dA
5.0		1.24 aC	1.36 aC	1.86 bB	2.53 cA
7.5		1.10 abD	1.40 aC	1.77 bcB	2.82 bA
10.0		0.97 bC	1.33 aC	2.31 aB	3.19 aA
12.5		0.99 bD	1.29 abC	2.19 aB	3.24 aA

Average followed by the same uppercase letter in the row and lowercase in the column does not differ by the Tukey test ($p < 0.05$).

Table 3. Shoot dry biomass (g) (SDB), root dry biomass (g) (RDB), total dry biomass (g) (TDB), height/diameter ratio (H/D), and Dickson Quality Index (DQI) of *Berberis laurina* seedlings submitted to increasing controlled-release fertilizer [Basacote® Plus 9M (16-8-12)] doses at 150 days.

Doses (Kg m-3)	SDB	RDB	TDB	H/D	DQI
		(g)			
0.0	0.24 d	0.28 d	0.51 d	2.28 d	0.16 d
2.5	4.43 c	1.91 c	6.35 c	4.66 c	0.91 c
5.0	5.49 c	2.09 c	7.59 c	5.89 bc	0.91 c
7.5	11.32 b	3.53 b	15.23 b	7.624 a	1.39 b
10.0	11.69 b	4.07 b	15.39 b	7.58 a	1.47 b
12.5	14.84 a	5.56 a	20.39 a	7.44 ab	2.02 a

Average followed by the same letter in the column does not differ by the Tukey test ($p < 0.05$).

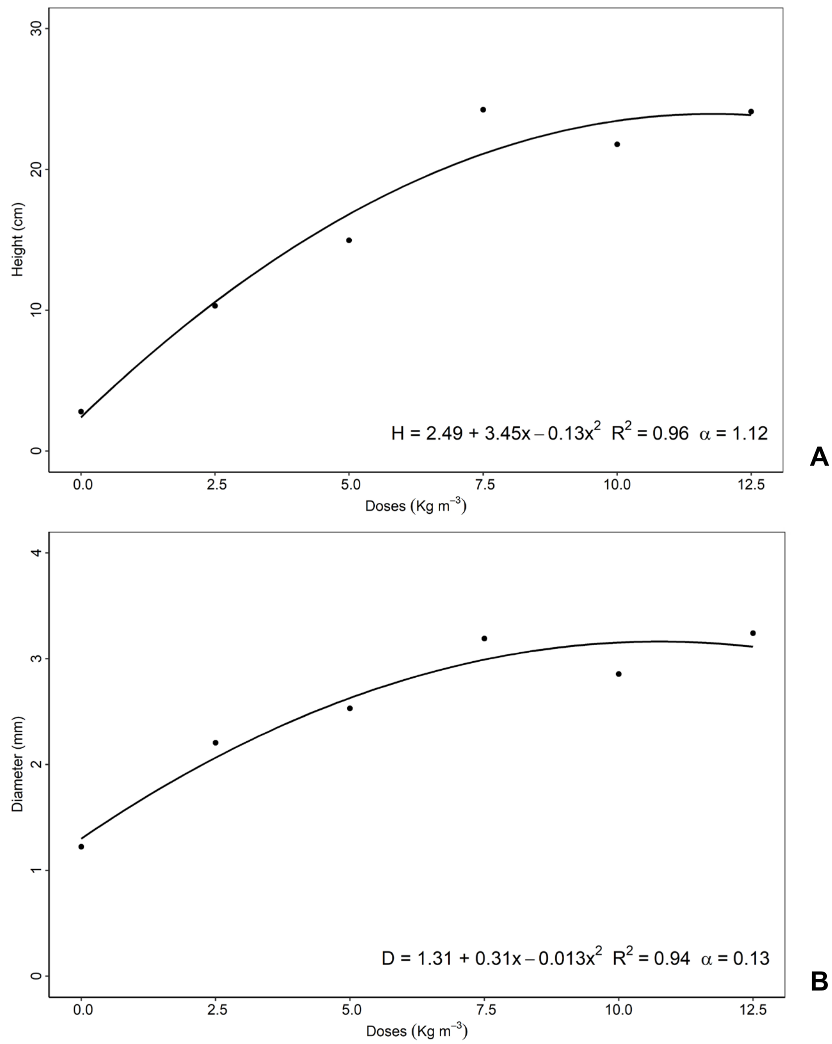

Figure 1. Polynomial regression for height (H) and diameter (D) of *Berberis laurina* Billb. seedlings submitted to increasing controlled-release fertilizer [Basacote® Plus 9M (16-8-12)] doses at 150 days. α = standard error; R^2 = adjusted r.

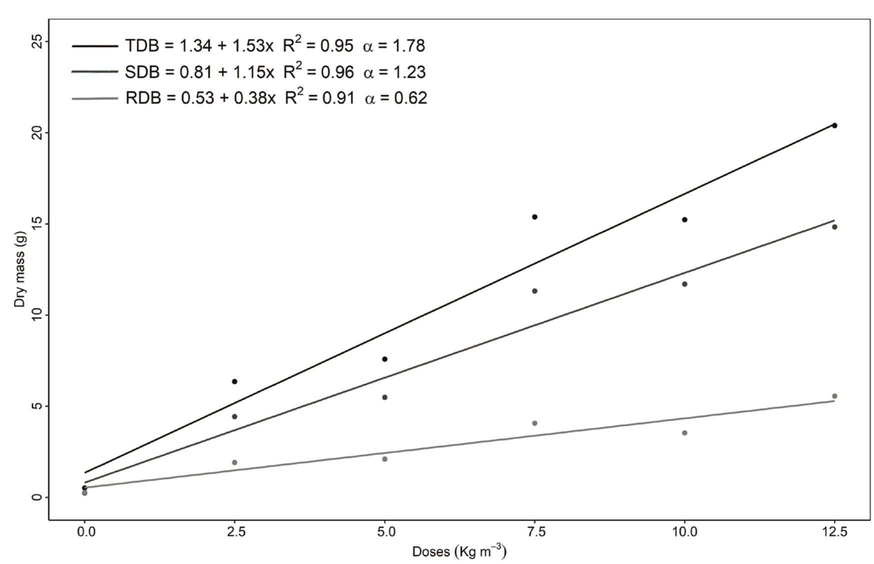

Figure 2. Linear regression for shoot dry biomass (SDB), root dry biomass (RDB), and total dry biomass (TDB) of *Berberis laurina* Billb. seedlings submitted to increasing controlled-release fertilizer [Basacote® Plus 9M (16-8-12)] doses at 150 days. α = standard error; R^2 = adjusted r.

Figure 3. Comparative of *Berberis laurina* Billb. seedlings submitted to increasing controlled-release fertilizer [Basacote® Plus 9M (16-8-12)] doses at 150 days.

DISCUSSION

It is common, in forest nurseries, to evaluate seedling quality only at the end of the pre-established production period. However, this evaluation can occur in several stages of production as a monitoring method. These measurements provide important information about seedling quality (Grossnickle & MacDonald 2018), or even ensure the end of production ahead of schedule. In this case, from 60 days of *B. laurina* seedlings production, it would be possible to distinguish plants with poorly supplied needs, such as the lack of nutrients needed for their growth. After 150 days in the nursery, only plants produced with 7.5, 10.0 and 12.5 kg m^{-3} of CRF would be suitable for field planting considering parameters established for other species (Binotto *et al.* 2010, Silva *et al.* 2014, Brito *et al.* 2018, Cabreira *et al.* 2019). CRFs provide nutrients to plants slowly, enabling the reduction of losses and ensuring essential elements according to the need in each phase of seedlings production in the nursery (Santos *et al.* 2020). However, results obtained up to 30 days after the CRF application indicate that there is time until plants adapt after the picking and nutrients release is established so that seedlings start to grow properly.

Height and diameter are good parameters for determining seedling quality because they are non-destructive and easy to measure (Ivetić *et al.* 2016, Grossnickle & MacDonald 2018, Khanal *et al.* 2018). At the end of the production period, we observed the highest values in plants with 10.0 and 12.5 kg m^{-3} of CRF. Studies on the establishment and initial growth of *Berberis* seedlings indicate the high plasticity of species under several environmental factors (McAlpine & Jesson 2007, Lubell & Brand 2011, Arena *et al.* 2020). As the aforementioned authors concluded, this plasticity can be advantageous under conditions of nutrients excess in the substrate – *Berberis* plants develop satisfactorily even when other species would present toxicity and a reduction in growth rate. However, it is important to pay attention to the costs of resources excess considering productivity increase.

Biomass accumulation in seedlings is an important indicator of seedling growth as it directly reflects the positive result of photosynthesis energy balance (Santos *et al.* 2020). Biomass distribution, in turn, is closely related to the seedling establishment and initial growth in the field (Binotto *et al.* 2010). Excess of nutrients during seedling production may result in greater biomass allocation in the aerial part, as we observed in this study. High SDB with low

RDB may be unfavorable in the field because it provides greater water loss through transpiration (Cortina *et al.* 2013). In a study on *Berberis darwinii* Hook. establishment and development under different light availabilities, the authors observed a higher biomass allocation in plants aerial part than in the root system (McAlpine & Jesson 2007). The increase in biomass/leaf area ratio according to nutrient availability was observed in *Berberis macrophylla* G. Frost., with significant differences also for chlorophyll *a*, *b* and carotenoids contents (Arena *et al.* 2020). Nutrient accumulation, especially phosphorus, directly reflects on plant response in the field, stimulating root growth and ensuring a greater area of water and soil nutrient absorption (Dias *et al.* 2017).

The growth indices used in this study indicate that treatments with 7.5, 10.0, and 12.5 kg m^{-3} of CRF produced seedlings suitable for field planting. It is important to note that these quality indicators vary according to specie intrinsic characteristics, and this should be considered when establishing quality parameters. We must also consider environmental conditions as a determining factor for the H/D ratio adequating values, so this is not a fixed parameter for all forest species quality (Grossnickle & MacDonald 2018). DQI considers the morphological characteristics most commonly measured and with well-established relationships with plant performance in the field (Binotto *et al.* 2010); therefore, it is a more reliable parameter in determining seedlings quality. In this study, we can consider H/D values between 5.0 and 8.0 and DQI values between 1.0 and 2.0 to be adequate.

In forest seedlings production, we must take into account the species best response to fertilizer availability (Mikula *et al.* 2020), so that there are no unnecessary costs or decreases in seedlings development of seedlings in the field. In this study, we observed that high doses of nutrients may be beneficial for *B. laurina* seedlings without providing a significant increase in productivity. Our results will be fundamental for the proper establishment of *B. laurina* seedlings production at a commercial level – an essential resource for the development of products related to their medicinal and ecological potential.

The doses 7.5, 10.0, and 12.5 kg m^{-3} of CRF provide *B. laurina* seedlings adequate growth, reaching the recommended parameters for field planting after 150 days. Considering the best use of resources in forest seedling production, we recommend 7.5 kg m^{-3} of CRF for this species seedlings production.

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