Vol.65: e22210394, 2022
https://doi.org/10.1590/1678-4324-2022210394
ISSN 1678-4324 Online Edition

Article - Agriculture, Agribusiness and Biotechnology

Growth and Quality of Yerba Mate Seedlings Affected by Fertilizer Doses in South Brazil

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Adriel Ferreira da Fonseca

Received: 14-Jun-2021; Accepted: 24-Fev-2022.

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HIGHLIGHTS

- Yerba mate seedlings are growth with controlled-release fertilizer.
- Use of controlled-release fertilizer reduces the production costs.
- The fertilizer 3M has a lower input required than fertilizer 9M.

Abstract: Given the lack of information on the use of controlled-release fertilizers in yerba mate seedlings production, this study aimed to evaluate growth and morphological characteristics of seedlings produced with two controlled-release fertilizers applied at different doses. We used seeds from a clonal seed orchard, which were sown in a commercial substrate based on peat and vermiculite, with Osmocote® MiniPrill 3M (CRF 3M) and Basacote® Plus 9M (CRF 9M) fertilizers, both in doses of 0, 2, 4, 6, 8, 10, and 12 kg m^{-3}. Seedling height and stem diameter were measured at 60, 90, 120, and 180 days after sowing and, at 180 days, shoot, root, and total biomass, tube withdrawal ease, and root aggregation. From these data, we calculated Dickson's quality index, technical efficiency, Height/Stem diameter ratio, and maximum technical efficiency dose. We verified values statistically superior to control treatment (0 kg m^{-3}) in all variables in response to controlled-release fertilizer 3M and 9M, demonstrating the viability of yerba mate seedling production using controlled-release fertilizers. This is also evidenced through results obtained for growth in height and stem diameter. Although CRF 3M favored seedling growth, CRF 9M showed similar efficiency. The Osmocote® MiniPrill 3M is more efficient than Basacote® Plus 9M in most of the variables analyzed and has a lower input required to obtain quality seedlings. For these reasons, Osmocote® MiniPrill 3M is indicated to produce yerba mate seedlings in the dose 8.50 kg m^{-3}.

Keywords: controlled-release fertilizer; *Ilex paraguariensis*; plant nutrition; seedling production; forest nursery.

INTRODUCTION

Belonging to the Aquifoliaceae family, *Ilex paraguariensis* A.St.-Hil. (yerba mate) is an arboreal species native to the Mixed Rainforest, with economic, social, and ecological importance, produced mainly by small properties in Southern Brazil [1-3]. Its use occurs through its leaves and fine branches to make drinks such as “chimarrão”, “tereré”, and teas and the production of cosmetics, medicines, and food, with an average consumption that can reach 10 kg $person^{-1}$ $year^{-1}$ [4]. In addition to South Brazil (in Paraná, Santa Catarina, and Rio Grande do Sul), yerba mate plantations are found in Mato Grosso do Sul and neighboring countries such as Argentina, Paraguay [5,6].

It is estimated that more than 15 million seedlings of yerba mate are produced annually in Brazil, mostly with seeds from native or cultivated stands, without any selection criteria [7]. The low genetic quality of seedlings, added to the absence of application, by producers, of a technical and technological package available for yerba mate plantations, has led to varied productivity results, with indications of average productivity of 7.7 t ha^{-1} [8,16]. However, the increase in cultivated areas in recent years was accompanied by increasing seedlings demand and, as a result, the development of research to improve practices and materials for establishing high-quality stands [9].

For the establishment of yerba mate stands, seedling production is one of the most important steps to guarantee quality [10] and field survival [11-13]. To obtain seedlings with qualitative characteristics that reflect greater vigor and growth after planting is the main challenge of forest nurseries engaged in yerba mate seedling production. Attributes such as seedling vigor, size and color of leaves, and good nutritional status are important factors influencing on initial survival and establishment of yerba mate plantations [10]. Another point is the increase in root density, which suggests adequate nutrients balance and storage, thus increasing resistance to stresses commonly faced in the field [14].

A critical factor for high-quality seedlings production refers to the nutrition made available to plants, which, when done correctly, allows the species to have a better performance both in nursery and in the field [9,15]. However, the availability of these nutrients should be taken into consideration so that there is no deficit or excess of available nutrients [11]. There is a great difficulty in developing specific fertilization protocols for each species. Among the available nutrients, studies show that yerba mate has a higher requirement for P>N>K>Mg>Ca, showing higher growth when using fertilizers that combine these nutrients [6,17]. In addition to the composition, the use of controlled-release fertilizers (CRF) has demonstrated for several species a better synchronization between plant physiological demands and nutrients availability [18-23], allowing for better use and lower leaching rate [24,25].

Given the variety of chemical fertilizers found in the agricultural market, there is a clear need for studies on this topic. Nutritional protocols must consider species characteristics to determine appropriate technical prescriptions. Based on this information, our study formulated the hypothesis that increasing doses of two commercial fertilizers with different nutritional compositions and release times will result in morphological differences in yerba mate seedlings. Thus, the present study aimed to evaluate growth and morphological characteristics of yerba mate seedlings produced with different doses of controlled-release fertilizers Osmocote® MiniPrill 3M and Basacote® Plus 9M.

MATERIAL AND METHODS

The experiment was carried out between November 2017 and April 2018 on the forest nursery of Bitumirim S.A. company, located in Ivaí, Paraná, Brazil (25º1'8.64'' S and 50º49'13.29'' W at 750 m altitude). According to the Köppen classification [26], region's climate is Cfa type, with an average temperature of the coldest month below 18 ºC, an average temperature of the hottest month above 22 ºC, and average precipitation around 1 500 mm.

We used the commercial substrate Carolina Soil Padrão®, based on peat and vermiculite. Different doses of controlled-release fertilizers of the brands Osmocote® MiniPrill 3M (CRF 3M), with a three-month release, and Basacote® Plus 9M (CRF 9M), with a nine-month release, were added to the substrate. The specifications for each fertilizer are shown in Table 1.

Table 1. Nutritional formulation of Osmocote® MiniPrill 3M (CRF 3M) and Basacote® Plus 9M (CRF 9M) controlled-release fertilizers.

Fertilizer	Macronutrients (%)					Micronutrients (%)					
	N	P_2O_5	K_2O	MgO	SO_4	Fe	B	Zn	Cu	Mn	Mo
CRF 9M	16	8	12	2	5	0.4	0.02	0.02	0.05	0.06	0.015
CRF 3M	19	6	10	-	3.5	-	-	-	-	-	-

N - Nitrogen; P_2O_5 - Phosphorus; K_2O - Potassium; MgO - Magnesium; SO_4 - Sulfur; Fe - Iron; B - Boron; Zn - Zinc; Cu - Copper; Mn - Manganese; Mo - Molybdenum.

The doses of 3M and 9M CRF were weighed on a precision scale (0.001 g) according to the following treatments: 0, 2, 4, 6, 8, 10, and 12 kg m^{-3}, each dose mixed with the substrate. We used seeds from a clonal seed orchard belonging to the company Bitumirim SA, which were stratified according to a methodology proposed by [27]. After the stratification process, seedlings with a root axis of up to 1.5 cm were sown in 110 cm^3 tubes and covered with a layer of ground coconut fiber.

After sowing, trays were placed in a greenhouse with irrigation by nebulization at intervals of 1 hour, lasting 60 seconds, and a 28 L $hour^{-1}$ flow rate. After 30 days, irrigation started to be applied in intervals of three hours to supply the needs of seedlings in more advanced growth stages. After 105 days, seedlings were transferred to a shade house (70% shading) with irrigation by micro-sprinkler, with four daily irrigations lasting 10 minutes and a flow rate of 144 L $hour^{-1}$, where they remained until the end of experiment.

During the experimental period, we evaluated shoot height (with a millimeter ruler) at 60, 90, 120, and 180 days, and stem diameter (with a digital caliper) at 90, 120, and 180 days. At 180 days, four plants per sampling unit were selected to assess shoot, root, and total dry biomass, tube withdrawal ease (TWE), and root aggregation to the substrate (AG), following a methodology described by [28]. Dry biomass was obtained by drying the material in an oven for 48 hours at 65 °C, with subsequent weighing in an analytical balance with 0.001 g precision. From these data, we calculated a morphological ratio index between height and stem diameter (H/D), and Dickson quality index (DQI), using the following Formula 1:

$$DQI = TDB / ((H / D) + (SDB / RDB)) \quad (1)$$

Where: H = shoot height (cm), D = stem diameter (mm), TDB = total dry biomass (g), SDB = shoot dry biomass (g) e RDB = root dry biomass (g).

In the end, we calculated a qualitative index called technical efficiency (Te), in which the highest value represents the most appropriate treatment and the lowest value the least suitable for yerba mate seedlings production, based on the study developed by [29], according to Formula 2:

$$Te = (TDB \times 0.40) + (SDB \times 0.25) + (D \times 0.10) + (H \times 0.05) + (AG \times 0.05) \quad (2)$$

Where: TDB = total dry biomass (g), SDB = shoot dry biomass (g), D = stem diameter (mm), H = shoot height (cm), AG = root aggregation to the substrate.

The experiment was carried out following a completely randomized design (CRD). For height and stem diameter, the Split-Plot design overtime was used, with four replications of 20 seedlings per experimental unit. For variables height/diameter ratio (H/D), tube withdrawal ease (TWE), root aggregation (AG), Dickson Quality Index (DQI), and technical efficiency (Te), analyzes were performed following a factorial scheme 2 x 7 (two CRFs x seven doses), with four seedlings for destructive variables (dry biomass). In both cases, the variances were subjected to Bartlett homogeneity test and Shapiro-Wilk normality test, and variables that showed significant differences ($p < 0.05$) in the analysis of variance (ANOVA) had their means compared by Scott-Knott test ($p < 0.01$ and $p < 0.05$). When the assumptions of homogeneity and normality were not observed, data were transformed by square root. Statistical analysis and graphs were performed with the aid of tidyverse multcomp and emmeans packages in R software [30].

The height, stem diameter, aerial, root, and total dry biomass variables were also subjected to polynomial regression analysis and, whenever possible, the maximum technical efficiency doses (MTED) were calculated using formula 3:

$$MTED = -b / 2a \quad (3)$$

Where: b and a = Coefficients of adjusted equation.

RESULTS

The analysis of variance revealed a significant interaction between CRF dose and evaluation periods for height and stem diameter in yerba mate seedlings for both fertilizers (3M and 9M) (Figure1). Especially from the dose 4 kg m^{-3}, statistically higher values for height and diameter were observed at 180 days, compared to other periods. In the control treatment (0 kg m^{-3} CRF), we did not observe a significant difference for height between measurements; however, we observed differences between 90 and 120 days, with a growth rate greater than 50%. For diameter, however, the greatest increase was obtained between evaluations at 90 and 180 days.

The fertilizer doses that provided higher values in height and diameter for yerba mate seedlings at the end of the experimental period (180 days) were 6, 8, 10, and 12 kg m^{-3} CRF 3M and 12 kg m^{-3} CRF 9M (Figure 1).

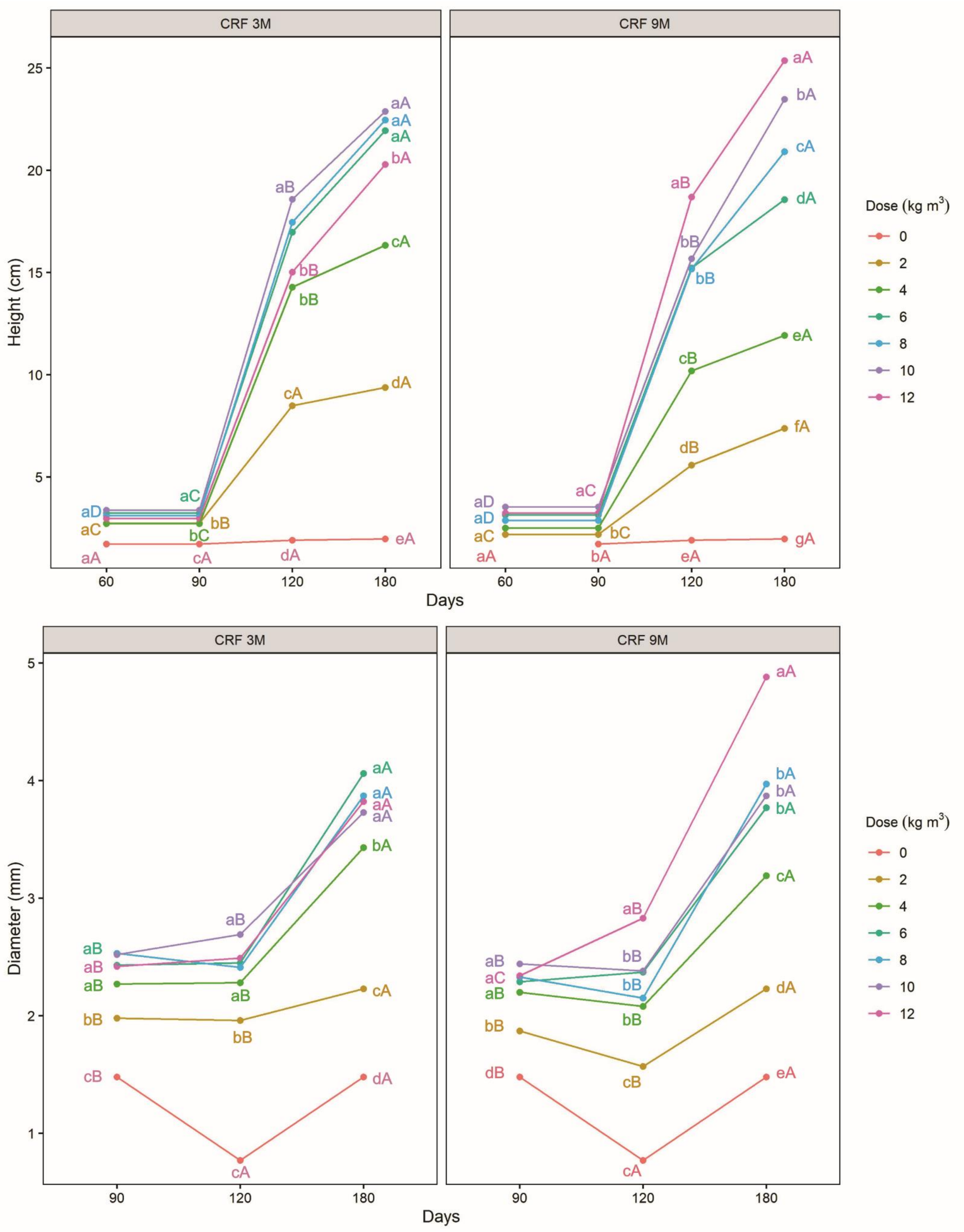

Figure 1. Height (H) and stem diameter (D) of *I. paraguariensis* seedlings produced under different doses of controlled-release fertilizers (CRF) Osmocote® MiniPrill 3M and Basacote® Plus 9M at 60, 90, 120, and 180 days after sowing. Lowercase letters corresponding comparison at same Day, uppercase letters corresponding comparison at Doses.

Regarding the other analyzes performed at 180 days, only variables Dickson quality index (DQI) and technical efficiency (Te) showed a significant interaction between fertilizers (3M and 9M) and applied doses (Table 2). For height/diameter ratio (H/D), tube withdrawal ease (TWE), and root aggregation (AG), there was no interaction. In general, numerically higher values for CRF 3M were verified in all variables, except for TWE; however, only in H/D ratio, AG, and Te differences were statistically significant. Regarding doses, results were quite different according to each variable analyzed. For variables DQI and Te, higher values were obtained with CRF 3M in the doses 10 kg m^{-3} for DQI (2.46) and 8 and 10 kg m^{-3} for Te (3.83 and 3.69, respectively), with a reduction from dose 10 kg m^{-3}. Differently for CRF 9M, DQI and Te significantly increased in response to an increase in doses with the highest averages obtained at 12 kg m^{-3} (2.41 and 3.78, respectively).

Table 2. Means of height/diameter ratio (H/D), tube withdrawal ease (TWE), root aggregation (AG), Dickson quality index (DQI), and technical efficiency (Te) of *I. paraguariensis* seedlings produced under different doses of controlled-release fertilizers (CRF) Osmocote® MiniPrill 3M and Basacote® Plus 9M, 180 days after sowing.

			H/D				
CRF	0 kg m^{-3}	2 kg m^{-3}	4 kg m^{-3}	6 kg m^{-3}	8 kg m^{-3}	10 kg m^{-3}	12 kg m^{-3}
3M	1.25 aA	3.81 aA	4.78 aA	5.61 aA	5.86 aA	6.14 aA	5.30 aA
9M	1.25 aA	3.51 aA	3.83 aA	4.92 aA	5.27 aA	6.09 aA	5.23 aA
CV % = 9.56							
			TWE*				
3M	0.00 aA	3.03 aA	2.96 aA	2.97 aA	2.92 aA	2.98 aA	2.96 aA
9M	0.00 aA	2.93 aA	3.02 aA	2.95 aA	2.97 aA	2.98 aA	3.00 aA
CV % = 2.74							
			AG				
3M	0.00 aA	8.47 aA	9.05 aA	9.35 aA	9.70 aA	9.30 aA	8.95 aA
9M	0.00 aA	6.80 aA	8.15 aA	8.50 aA	8.85 aA	8.20 aA	8.95 aA
CV % = 7.53							
			DQI				
3M	0.00 aD	1.01 aC	1.57 aB	1.79 aB	2.46 aA	1.94 aB	1.66 bB
9M	0.00 aD	0.67 aC	1.02 bC	1.40 aB	1.89 bB	1.62 aB	2.41 aA
CV % = 14.25							
			Te				
3M	0.13 aE	2.12 aD	2.79 aC	3.30 aB	3.83 aA	3.69 aA	3.18 bB
9M	0.13 aF	1.69 bE	2.24 bD	2.79 bC	3.31 bB	3.22 bB	3.78 aA
CV % = 10.48							

Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by the Scott-Knott test at 5 % probability.
* Original data transformed by square root.

After adjusting the equations, all parameters showed quadratic behavior (Figure 2). For CRF 3M, height, stem diameter, dry biomass, and DQI showed an inflection point from 10 kg m^{-3}; treatments with CRF 9M maintained the upward growth for height and diameter and the beginning of an inflection point for other variables. Thus, the doses of maximum technical efficiency (DMTE) verified for CRF 3M were 8.62 kg m^{-3} and 8.64 kg m^{-3} for height and diameter, respectively, 8.48; 8.06; 8.59 for shoot, root, and total dry biomass, respectively, and 8.59 kg m^{-3} for DQI. With these values, there is an average dose of maximum efficiency of 8.50 kg m^{-3} for CRF 3M. For CRF 9M, there was a greater variation concerning the calculated DMTE values, obtaining for shoot, root, and total dry biomass, doses 11.43; 10.63; 11.41 kg m^{-3}, respectively, and 10.66 kg m^{-3} for DQI. Thus, the average dose of maximum efficiency for CRF 9M would be 11.15 kg m^{-3}.

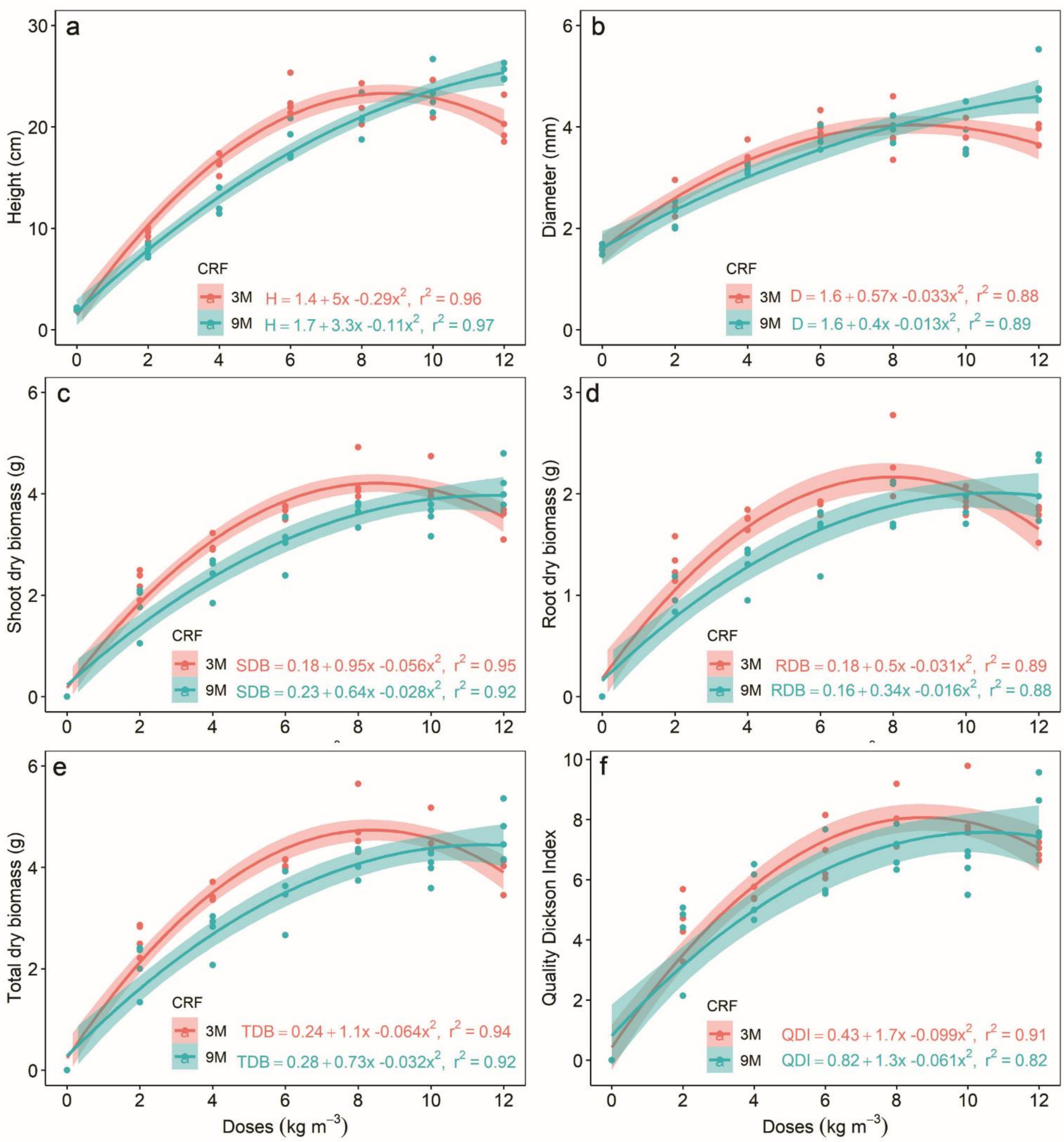

Figure 2. Polynomial regression adjusts for (a) height, (b) stem diameter, (c) shoot dry biomass, (d) root dry biomass, (e) total dry biomass, and (f) Dickson quality index in *I. paraguariensis* seedlings submitted to different doses of controlled-release fertilizers (CRF) Osmocote® MiniPrill 3M and Basacote® Plus 9M, 180 days after sowing.

At the points where both fertilizer curves are similar, were obtained for dry biomass and DQI between the doses 10 and 12 kg m^{-3}. For stem diameter, this behavior was observed at the dose 8 kg m^{-3} and for height at the dose 10 kg m^{-3} CRF.

DISCUSSION

Results statistically superiors to the control treatment for all variables in response to fertilizers demonstrate the viability of a seedlings production protocol for yerba mate using controlled-release fertilizers. It is evidenced throughout results obtained for growth in height and diameter (Figure 1). The use of controlled-release fertilizers can be a positive factor concerning seedling production costs since their incorporation into the substrate, without the need for further applications, reduce operating costs [19]. Also, it can alleviate problems caused by nutritional deficits throughout seedling production cycle, especially in production models with low technological levels.

Concerning different CRF doses, the values frequently higher for height and stem diameter in response to an increase in doses reinforce results that point to better seedling development under greater nutrients availability [6,31]. According to some authors, seedling quality has a high correlation with height and stem diameter, generating higher rates of survival and growth in the field [32,34] and, consequently, higher financial returns than those obtained through low-quality seedlings [35]. Thus, several studies have indicated the use of fertilizer to improve growth of yerba mate seedlings, mainly through N, P, and K availability [6,17,31]. These nutrients are required by plants in quantities that vary according to species and development stage; however, both deficiency and excess can be harmful to plant development [36]. Nutrient excess was possibly caused in yerba mate seedlings submitted to 12 kg m^{-3} CRF 3M. In this treatment, from 120 days after sowing, we observed a reduction in height values comparing to values obtained for 10 kg m^{-3} (Figure 1). Nutrient excess in yerba mate seedlings also reinforced results that point to biomass reduction, differently from results obtained without fertilizers, which represent a lack of nutrients.

It is known that, in seedling production, nutrients excess symptoms are expressed in a less specific way than deficiency symptoms, except when toxicity generated by one element induces a deficiency of another [37]. Phosphorus in excess, for example, can compromise plant nutrition when interacting in an antagonistic way with micronutrients such as Zn, Fe, Mn, and Cu, without necessarily having a decrease in these micronutrients content in the plant [38]. Likewise, potassium in excess can interfere with plant absorption of other cations, inhibiting, for example, the absorption of Ca and Mg [39]. Nitrogen excess can promote excessive stem growth, causing an imbalance between shoot and root system, reducing tissue lignin content and seedling resistance in the nursery, and increasing susceptibility to pests and diseases, and less resistance to field conditions [40].

Despite the reduction in values observed for CRF 3M from dose 10 kg m^{-3}, this fertilizer was superior to CRF 9M in most variables analyzed for yerba mate. Other attributes such as H/D ratio, tube withdrawal ease, and root aggregation are important for defining seedling quality [9] and reflect on quality indexes such as DQI and Te. Thus, the lowest averages obtained by CRF 9M for H/D ratio and AG could represent a lower potential for yerba mate seedling production. However, it follows values frequently observed for these variables [9], and only the dose to be applied should be reconsidered. For H/D ratio, means obtained with 4 and 6 kg m^{-3} for CRF 3M and 9M, respectively, are considered ideal for high-quality seedlings. Doses above 10 kg m^{-3} can generate a greater susceptibility of seedlings to tipping in the field, due to high etiolation and reduced stem resistance [41,42], in addition to difficulty in absorbing and transferring water to upper portions, impairing initial establishment [43]. Likewise, values obtained for root aggregation with 6 kg m^{-3} of both fertilizers are considered adequate and reflect a good root system development [9]. In this case, increasing root aggregation leads to greater chances of plant establishment and initial development in the field.

The higher averages for the CRF 3M, in any case, is related to greater nitrogen availability in this fertilizer, with composition NPK 19-6-10, compared to CRF 9M, with NPK 16-8-12, and to the shorter nutrient release time. Nitrogen is an important macronutrient for vegetables, and like other elements, when applied at correct doses, it can favor the maximum seedling growth potential [17]. These results are reinforced by height, stem diameter, and dry biomass in polynomial regression analysis (Figure 2), which is related to the period of nutrient release from fertilizers to seedlings. Therefore CRF 3M, by faster-releasing nutrients, enabled a greater yerba mate seedling growth comparing to CRF 9M. Thus, CRF 9M to reach seedling maximum growth potential, requires a higher dose. On the other hand, CRF 3M applied in doses above 8.50 kg m^{-3} – average dose of maximum efficiency for the analyzed variables – tends to reduce seedling development due to a phytotoxic effect already reported. Different results regarding fertilizers efficiency and periods of nutrient release demonstrate that not only fertilizer composition is important for plant development, but also release time determines what nutrients are available during seedling growth.

Regarding DQI, which considers the biomass of different plant parts, height, and stem diameter, the maximum values obtained for the two fertilizers were similar, 2.46 and 2.41 for CRF 3M and 9M, respectively. This growth ratio differed only in dose (Table 2), possibly related to fertilizer release time, as already highlighted through the polynomial regression analysis (Figure 2). Therefore, the application of CRF 9M higher doses could promote better results since we observed an increase in values in response to increasing doses. Although it is not certain that seedling development in nursery will reflect its development in the field, several studies reinforce the use if these indexes to predict seedling responses to planting stress and the ability to adapt to field conditions [10,13], reducing the need for replanting and post-planting maintenance costs [21].

Thus, when we relate growth variables to DQI and Te quality indexes, there is an indication that CRF 3M would be the best option for yerba mate seedlings production. A smaller input of this fertilizer would be necessary to obtain seedlings with adequate quality and growth patterns. Also, considering the costs of these fertilizers, CRF 3M is sold for an average price of US$ 161,20 kg^{-1}, and CRF 9M for US$ 184,80 kg^{-1}, both quoted in June 2020. The values were transformed from real (R$) to dollar (US$), based on the exchange rate at this time (US$ 5,28). From the average values obtained for the doses of maximum technical efficiency, it was found that about 8.50 kg m^{-3} of CRF 3M is needed, against 11.15 kg m^{-3} of CRF 9M, representing an average cost of US$ 1363,93 for the CRF 3M and US$ 2055,55 for the CRF 9M.

Finally, the increasing use of fertilizers associated with a better seedling performance needs to adjust according to species and production cycle. In this case, its effects are beneficial to plants and guarantee a better field development without financial losses [44]. In the case of controlled-release fertilizers, some advantages refer to a reduction in labor used in fertigation [45], necessary for the periodic application of common fertilizers. CRF provides better use of nutrients due to the slow availability to the root system, minimizing risks of nutritional loss and deficiency, and may also reduce the production cycle [46]. We also emphasize that periodic fertilization by common fertilizers can be particularly difficult in the case of forest nurseries that are not dedicated to the production of a single species and, for this reason, have different fertilization protocols. Fertilization is so important that several studies have pointed out that regardless of the species, fertilizer application is the factor of greatest influence to accelerate plant growth and at the same time guarantee superior characteristics of vigor, resistance, and robustness [47,48]. With a well-formed shoot and root system, plants are more efficient at absorbing and using nutrients available at planting, resulting in greater field survival, and reduced replanting costs. Furthermore, in addition to the advantages of using controlled-release fertilizers, production costs must also be considered when choosing a fertilizer as economic viability is an important factor in decision making for seedlings production.

CONCLUSION

Both Osmocote® MiniPrill 3M and Basacote® Plus 9M fertilizers are suitable for yerba mate seedlings production.

The Osmocote® MiniPrill 3M fertilizer is more efficient than Basacote® Plus 9M in most of the variables analyzed and has a lower amount of input required to obtain quality seedlings, indicating a dose of 8.50 kg m^{-3}. For the Basacote® Plus 9M fertilizer, a dose of 12.42 kg m^{-3} is suggested.

Acknowledgments: The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the doctoral scholarships and to Chimarrão Bitumirim Indústria e Comércio de Erva-mate Ltda for technical and logistical support.
Conflicts of Interest: The authors declare no conflict of interest.

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