

PERIODS OF COLLECTION AND SIZE OF MINI-CUTTINGS IN YERBA-MATE ADVENTITIOUS ROOTING

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Received for publication: 20/01/2022 – Accepted for publication: 12/06/2023

Resumo

Períodos de coleta e tamanho de miniestacas no enraizamento adventício de erva-mate. Para aperfeiçoar a produção de mudas de erva-mate a partir do aumento das taxas de enraizamento, lança-se mão da propagação clonal, em especial a técnica de miniestaquia, que visa aumentar os ganhos genéticos e de eficiência na produção. Desta forma objetivou-se avaliar a influência de diferentes tamanhos de miniestacas e coletas sucessivas sobre o enraizamento e vigor radicial de *Ilex paraguariensis*. Foram realizadas quatro coletas sucessivas e confeccionadas miniestacas de 4, 6, 8 e 10 cm ($\pm 0,2$) de comprimento, as quais foram estaqueadas em tubetes de 110 cm³ e mantidas em casa de vegetação climatizada por 45 dias. Foram considerados o percentual de enraizamento, número de raízes miniestaca⁻¹, comprimento médio das três raízes maiores miniestaca⁻¹, calos, mortalidade, sobrevivência e manutenção de folhas originais. O enraizamento foi influenciado pelo tamanho das miniestacas, sendo tamanhos de 8 cm e 10 cm tenham apresentado maior vigor. A boa adaptação das minicepas tende a favorecer o enraizamento das miniestacas ao longo das coletas, evidenciando o potencial da técnica de miniestaquia para a influência vegetativa de *Ilex paraguariensis*.

Palavras-chave: *Ilex paraguariensis*, propagação vegetativa, qualidade radicial, qualidade morfológica.

Abstract

Periods of collection and size of mini-cuttings in yerba-mate adventitious rooting. To improve the production of yerba mate seedlings by increasing rooting rates, clonal propagation is used, especially the mini-cutting technique, which aims to increase genetic gains and production efficiency. Thus, the objective was to evaluate the influence of different sizes of mini-cuttings and successive collections on the rooting and root vigor of *Ilex paraguariensis*. Four successive collections were carried out and mini-cuttings measuring 4, 6, 8 and 10 cm (± 0.2) in length were made, which were staked in 110 cm³ tubes and kept in a climate-controlled greenhouse for 45 days. Rooting percentage, number of mini-cutting roots⁻¹, average length of the three largest mini-cutting roots⁻¹, callus, mortality, survival, and maintenance of original leaves were considered. Rooting was influenced by the size of the mini-cuttings, with mini-cuttings of 8 cm and 10 cm showing greater vigor. The good adaptation of the mini-stumps tends to favor the rooting of the mini-cuttings throughout the collections, showing the potential of the mini-cutting technique for the vegetative influence of *Ilex paraguariensis*.

Keywords: *Ilex paraguariensis*, vegetative propagation, rooting, morphological quality.

INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St.-Hil.) is a species native to the Mixed Ombrophylous Forest, naturally occurring in southern Brazil, Paraguay, and Argentina (CARVALHO, 2003). It is one of the main forest products for non-timber purposes sold in Brazil, producing approximately 500 tons in 2021 (IBGE, 2022). Its primary form of consumption is as “chimarrão” and teas, but its leaves are used for dyes, sweets, ice cream, caramels, medicines, and perfums (DARTORA *et al.*, 2013).

Despite its importance for the economy of South America, mate plantations still face limitations. Most of the seedlings comprising the species' plantations are of seminal origin, with seeds of low genetic quality (SANTIN *et al.*, 2008) and low germination vigor. Seminal plantations exhibit high genetic variability, which decreases the productivity and quality of the final product (WENDLING; SANTIN, 2015). On the other hand, clonal plantations possess genetic superiority, which can enhance the productivity and quality of the final product. Therefore, vegetative propagation emerges as an alternative to sexual propagation and its limitations. It is possible to multiply superior genotypes and retain genotypic characteristics of interest through vegetative propagation (XAVIER *et al.*, 2013). Among the asexual propagation techniques, mini-cutting stands out, involving the maintenance of plants in a mini-garden system to produce shoots that will give rise to mini-cuttings. The literature demonstrates that mini-cutting is a promising technique for yerba mate propagation (GAZZANA *et al.*, 2019; SÁ *et al.*, 2018; PIMENTEL

et al., 2019; DUARTE *et al.*, 2020); however, the rooting percentages obtained so far for yerba mate mini-cuttings are low (60% on average), requiring further studies on their rhizogenic process.

Currently, several nurseries adopt the mini-cutting protocol established for *Eucalyptus* spp., which includes parameter such as a length of 4-8 cm, a beveled base cut, and leaf area reduction, among other. However, each species has its specific requirements, and the development of specific protocols for the asexual propagation of yerba mate is crucial to enhance mini-cutting production and increase rooting percentages. Therefore, our objective was to assess the impact of different mini-cutting sizes and successive collections on the rooting and root vigor of *Ilex paraguariensis*, with the aim of contributing to the development of an efficient propagation protocol.

MATERIAL AND METHODS

The experiment was conducted from October 2017 to March 2018 at Bitumirim Ind. e Com. de Erva-Mate Ltda., located in Ivaí, Paraná, Brazil (25°1'8.64"S and 50°49'13.29", 750 m). The mini-garden was established with seminal seedlings from a clonal seed orchard, cultivated in 110 cm³ tubes for approximately six months. The seedlings were planted in a semi-hydroponic system on a sand bed, with a spacing of 15 cm x 15 cm between plants. The mini-garden was set up inside a greenhouse covered with polyethylene, UV filter, and 50% shading. Drip fertigation was applied three times a day, with an average flow of 6 L m⁻² day⁻¹. The nutrient solution consisted of monoammonium phosphate (0.065 g L⁻¹), magnesium sulfate (0.40 g L⁻¹), potassium nitrate (0.44 g L⁻¹), ammonium sulfate (0.2 g L⁻¹), potassium sulfate (0.07 g L⁻¹), calcium chloride (0.40 g L⁻¹), boric acid (2.88 mg L⁻¹), manganese sulfate (3.70 mg L⁻¹), sodium molybdate (0.18 mg L⁻¹), zinc sulfate (0.74 mg L⁻¹), and hydroiron powder (81.80 mg L⁻¹), replaced every two weeks.

Apical pruning was carried out in the mini-garden to encourage orthotropic growth. Forty-five days after the establishment of the mini-garden, four consecutive collections were conducted at 30-day intervals, always performed selectively, retaining shoots less than 15 cm long for future collections. Mini-cuttings measuring 4 ± 0.2 cm, 6 ± 0.2 cm, 8 ± 0.2 cm, and 10 ± 0.2 cm in length, with an average diameter of approximately 0.4 ± 0.2 cm, were prepared with a bevel cut at the base and just above the last apical bud, keeping two leaves reduced to 50% of their original surface. Planting was done in 110 cm³ polypropylene tubes filled with a substrate of carbonized yerba mate and vermiculite (1/1 v/v). The mini-cuttings were planted 1 cm deep and kept in a greenhouse for 45 days with a temperature between 20 °C and 30 °C, relative humidity above 80%, and 50% shading.

After 45 days of experiment's establishment, we evaluated the following variables: percentage of rooting (live mini-cuttings with roots of at least 2 mm in length) (R), number of roots per mini-cuttings (NR), average length of the three largest roots per mini-cutting roots (cm) (ALLRM), percentage of mini-cuttings with calluses (live mini-cuttings without roots, with formation of undifferentiated cellular mass at the base) (C), percentage of dead mini-cuttings (mini-cuttings that presented necrotic tissue) (M), percentage of survival (live mini-cuttings that did not root or form calluses) (S), and leaf maintenance (mini-cuttings that maintained the original leaves in the root bed) (LM). The experiments were implemented in a completely randomized design, with four treatments (mini-cutting sizes) and five replications of 12 mini-cuttings per experimental unit. The first collection was in December (late spring), followed by subsequent collections in January, February, and March.

The data were subjected to the Bartlett test ($p < 0.05$) to assess the homogeneity of the variance, and to the normality test, with data transformations applied when necessary. Subsequently, analysis of variance (ANOVA, $p < 0.01$ and $p < 0.05$) was performed using a split-plot analysis over time. Depending on the significance of the ANOVA, factors (collection time and mini-cutting size) were further analyzed and compared using the Tukey test at a significance level of 5%. Correlation analysis between variables was conducted using Pearson's correlation analysis ($p < 0.01$ and $p < 0.05$). Data processing was conducted using R version 4.1 software (R Development Core Team, 2023).

RESULTS

The analysis of variance revealed a significant effect, as determined by the F-test ($p < 0.01$ and $p < 0.05$), for the interaction between "collection period x mini-cutting size" only in the case of the average length of the three largest root mini-cuttings, suggesting that the other analyzed variables are independent (Table 1). A significant effect was observed for the collection period in all variables, except for survival and the number of roots. Concerning the size of the mini-cuttings, it only influenced rooting, the number of roots, and the average length of the three largest roots. The survival rate of the mini-cuttings remained consistent across all experimental evaluations, with no significant differences observed.

Table 1- Summary of analysis of variance for rooting (R), number of roots minicuttings (NR), average length of the three largest roots (ALLRM), callus (C), mortality (M), survival (S) and leaf maintenance (LM) in minicuttings of *Ilex paraguariensis* A. St.-Hil.

Tabela 1 - Resumo da análise de variância para enraizamento (R), número de raízes (NR), comprimento médio das três maiores raízes (ALLRM), calos (C), mortalidade (M), sobrevivência (S) e manutenção foliar (LM) em miniestacas de *Ilex paraguariensis* A. St.-Hil.

Source of Variation	GL	Mean Squares						
		R ⁽¹⁾	C ⁽¹⁾	S	M ⁽¹⁾	LM ⁽¹⁾	NR	ALLRM
				%			mini-cutting ⁻¹	cm
Collecting	3	0.41**	0.34**	48.13 ^{ns}	0.21**	0.43**	0.76 ^{ns}	4.51**
Collecting residue	16	0.03	0.02	25.68	0.02	0.04	1.11	0.53
Size	3	0.11*	0.04 ^{ns}	44.19 ^{ns}	0.01 ^{ns}	0.03 ^{ns}	23.23**	4.70**
Collecting x size	9	0.02 ^{ns}	0.01 ^{ns}	25.90 ^{ns}	0.01 ^{ns}	0.02 ^{ns}	0.79 ^{ns}	0.63*
Residue size	48	0.02	0.03	35.97	0.01	0.02	1.15	0.28

* significant value by the F test ($p < 0.05$); **significant value by the F test ($p < 0.01$); ns value not significant, GL = degrees of freedom. (1) Data transformed by $Arccos \sqrt{n/100}$, where n = sample data in all formulas.

By analyzing the graphs, it was possible to identify that the larger mini-cuttings exhibited robust root development in the greenhouse, particularly the 8 cm mini-cuttings (90% rooting and a higher number of roots) (Figure 1). On the other hand, smaller mini-cuttings (4 cm) displayed lower rooting rates (81%) and fewer roots when compared to larger mini-cuttings.

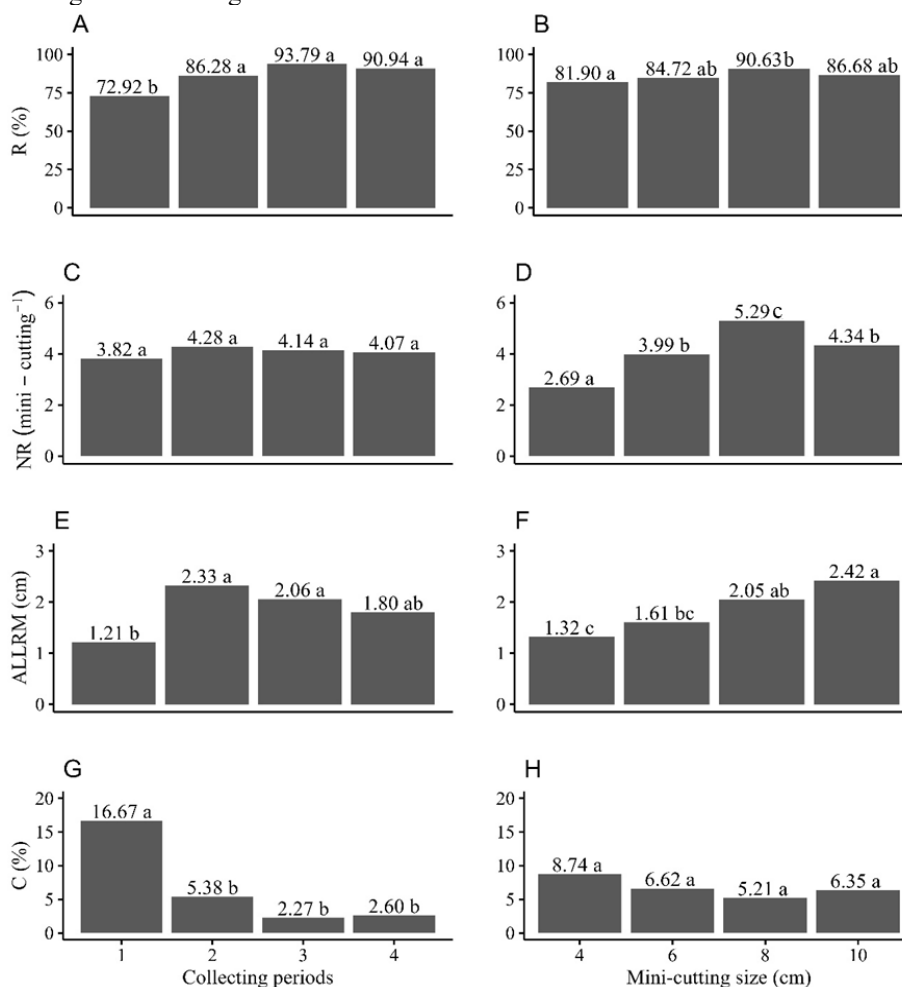


Figure 1 – Rooting (R), Number of roots (NR), Average length of the three largest root minicuttings (ALLRM) and Callus (C) in *Ilex paraguariensis* A. St.-Hil. mini-cuttings produced at four collection times and

four sizes. Means followed by the same letter do not differ statistically by Tukey's test at 5% significance.

Figura 1 – Enraizamento (R), Número de raízes (NR), comprimento médio das três raízes maiores (ALLRM) e Calos (C) em miniestacas de *Ilex paraguariensis* A. St.-Hil. produzidos em quatro coletas e quatro tamanhos. As médias seguidas da mesma letra não diferem estatisticamente pelo teste de Tukey a 5% de significância.

As the collections progressed, the mini-cuttings exhibited increased vigor, as indicated by the higher rooting rate (93% and 90% in the third and fourth collections, respectively), sustained leaf maintenance (above 97% from the third collection onward) and reduced percentage of calluses (2.27%) and mortality (0.62%) in the final collection (Figure 2).

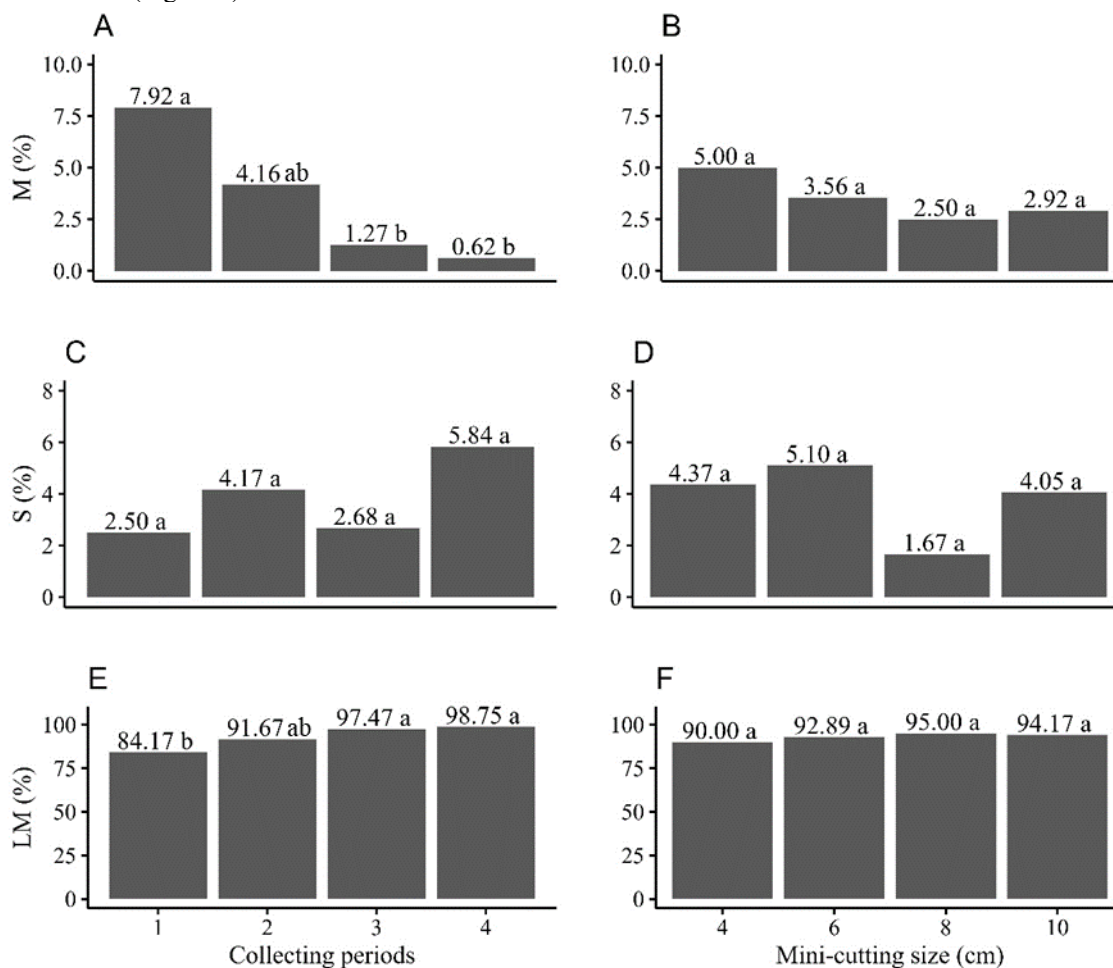


Figure 2 – Mortality (M), Survival (S) and Leaf maintenance (LM) in mini-cuttings of *Ilex paraguariensis* produced at four collection times and four sizes. Means followed by the same letter do not differ statistically by Tukey's test at 5% significance.

Figura 2 – Mortalidade (M), Sobrevivência (S) e Manutenção Foliar (LM) em miniestacas de *Ilex paraguariensis* produzidas em quatro tamanhos e quatro coletas. As médias seguidas da mesma letra não diferem estatisticamente pelo teste de Tukey a 5% de significância.

Only the length of the three largest roots correlates with the collection period and mini-cutting size; however, they follow the same pattern as the other analyzed variables. The longest roots were observed starting from the second collection, particularly in the 10 cm size (2.89% and 2.76%) during the third and fourth collections, respectively (Table 2).

Table 2 – Interaction between collecting periods and mini – cutting size of the average length of the three largest roots (ALLRM) in mini-cuttings of *Ilex paraguariensis* A. St.-Hil.

Tabela 2 - Interação entre período de coleta e comprimento médio das três raízes maiores (ALLRM) em miniestacas de *Ilex paraguariensis* A. St.-Hil.

Collecting Periods	ALLRM (cm)			
	4	6	8	10
1	0.80 Aa	0.95 Aab	1.33 Aab	1.75 Ab
2	2.28 Ba	2.24 Ba	2.52 Ba	2.25 ABa
3	1.25 Aa	1.82 ABab	2.23 ABbc	2.89 Bc
4	0.93 Aa	1.39 ABab	2.09 ABbc	2.76 Bc

Means followed by the same lowercase letters in the row and the same uppercase letters in the columns do not differ by Tukey's test ($p < 0.05$).

Through Person's correlation analysis, a positive correlation was identified between percentage of rooting and root vigor (represented by the variables number of roots and length of the three largest roots). Conversely, mortality and callus formation exhibited a negative correlation with the other variables, particularly with leaf maintenance (Figure 3).

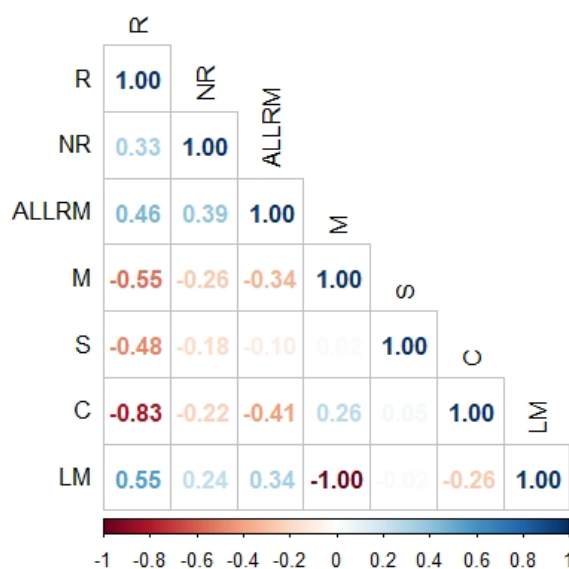


Figure 3 – Person correlation between rooting percentage (R), number of root mini-cuttings⁻¹ (NR), length of the three largest root mini-cuttings⁻¹ (ALLRM), calluses (C), mortality (M), survival (S), and leaf maintenance (LM) in *Ilex paraguariensis* mini-cuttings.

Figura 3 – Correlação de Person entre as variáveis enraizamento (R), número de raízes miniestaca⁻¹ (NR), comprimento médio das três raízes maiores miniestaca⁻¹ (ALLRM), calos (C), mortalidade (M), sobrevivência (S) e manutenção de folhas originais (LM) em miniestacas de *Ilex paraguariensis*.

DISCUSSION

The high rooting percentages of mini-cuttings (greater than 80%) obtained for all treatments over the four collections demonstrate the potential of the mini-cutting technique for the vegetative propagation of *Ilex paraguariensis*, as well as the material's strong adaptability to the environmental conditions of the nursery. Similar results were observed in other studies involving yerba mate mini-cuttings, considering various factors (STUEPP *et al.*, 2017; SÁ *et al.*, 2018; DUARTE *et al.*, 2020). Equally important is the proper management of the clonal mini-garden, particularly in terms of nutrient availability, which has been correlated with the enhancement of the ability to produce adventitious roots in woody species (ALMEIDA *et al.*, 2017). Adequate supplies of macro and micronutrients, primarily through nitrogen supplementation (ZERCHE; DRUEGE, 2009), are strongly associated with the rhizogenesis process (ALMEIDA *et al.*, 2017), a critical factor for the success of mini-cuttings (XAVIER *et al.*, 2013).

The superior rooting observed in larger mini-cuttings (6, 8, and 10 cm) is possibly linked to the greater quantity of plant hormones present in larger cuttings, promoting increased rooting vigor (ENGEL *et al.*, 2019). Another study involving the size of yerba mate propagules during the summer also reported improved results for rooting (86%), the number of roots (7.35), and leaf maintenance (98%) with larger mini-cuttings (10 cm), while callogenetic percentages and mortality decreased (DUARTE *et al.*, 2019, PIMENTEL *et al.*, 2021). However, research across different species, genetic materials, and types of propagules has yielded diverse results regarding the ideal mini-cutting size (SOUZA *et al.*, 2013; PONTES FILHO *et al.*, 2014; DUARTE *et al.*, 2019), justifying the need for studies like ours.

The low percentages of callus formation are associated with the high rooting percentages, as indicated by Pearson's correlation analysis. Direct root system formation in yerba mate has been observed in other studies, suggesting that callus formation is not essential for the development of adventitious roots (DUARTE *et al.*, 2019), as confirmed in our study. This result is advantageous for the vegetative propagation of yerba mate, as direct root system formation typically results in shorter greenhouse residence times and, consequently, higher cutting production. The low callus formation percentage may also be linked to the high juvenility of the propagules, considering their seminal origin in the mini-garden.

Similarly, the strong correlation observed in Pearson's analysis between rooting and leaf maintenance supports findings from other studies (DIAS *et al.*, 2012; FRAGOSO *et al.*, 2015; DUARTE *et al.*, 2020). Leaf maintenance is typically associated with successful vegetative propagation, as it serves as a vital source of carbohydrates and is involved in the synthesis of substances related to rhizogenesis (XAVIER *et al.*, 2013). While carbohydrates do not directly regulate rooting, they represent the energy available for synthesizing other essential substances in adventitious roots, potentially enhancing the effects of auxins, a hormone directly related to root development (KLOPOTEK *et al.*, 2012). This relationship was particularly evident in the first collection, where lower leaf maintenance resulted in lower percentages of adventitious rooting.

Regarding the different collection periods, some studies have noted that the collection timing directly influences the mini-cuttings' rooting process, with periods of milder temperatures tending to favor rooting (STUEPP *et al.*, 2017; SÁ *et al.*, 2018). However, we observed that the increase in rooting over successive collections was likely due to the improved adaptation of the mini-stumps to the mini-garden environment, as only the first collection exhibited lower rooting percentages (72.91%) compared to subsequent months (an average of 90.33%) during the summer period. A possible explanation for this improved rooting with successive collections may be the enhanced root development of the mini-stumps, leading to increased nutrient and carbohydrate absorption directly associated with mini-cutting rooting. Thus, higher reserve levels and a higher carbon-to-nitrogen ratio are likely to promote increased mini-cutting rooting (PELIZZA *et al.*, 2011).

CONCLUSION

- Different sizes of mini-cuttings have an impact on the rooting of *Ilex paraguariensis*, with mini-cuttings of 8 and 10 cm showing superior rooting and, consequently, better seedling vigor.
- Rooting of the mini-cuttings is consistently satisfactory in all summer collections, with a lower rooting percentage in the first collection, likely due to plant's acclimatization to the mini-garden environment.
- The mini-cutting technique demonstrate significant potential for the vegetative propagation of *Ilex paraguariensis* achieving rooting rates exceeding 93%.

ACKNOWLEDGMENTS

We dedicate this article to Nathalia Johanna Deen (*in memoriam*).

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