VEGETATIVE RESCUE AND PROPAGATION OF NATIVE *Ilex paraguariensis* POPULATIONS IN SANTA CATARINA STATE, BRAZIL

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ABSTRACT – When producing *Ilex paraguariensis* seedlings through vegetative propagation, selecting the correct populations and genotypes, and obtaining juvenile material, may be fundamental for its success. Therefore, this search aimed to test its vegetative propagation through cutting and rescue using detached branches of different populations in Santa Catarina state, Brazil. Thus, two experiments were installed in September 2019 in order to test: I) the cuttings of four populations belonging to the municipalities of Catanduvas (CT), Painel (PL), Três Barras (TB), and Urupema (UR), using ten randomly chosen genotypes from each, and; II) the epicormic sprouting of detached branches from these same populations. Both experiments were carried out in Lages, Santa Catarina. In February 2020, the cutting was evaluated according to the percentages of survival, callus, rooting, new sprouts, and original leaves permanence. The branches were evaluated every 30 days after storing, observing the percentages of sprouting branches, number of sprouts, and length of sprouts in centimeters. As for cutting, there was a low survival percentage in all populations (<15%), but some genotypes presented greater vigor, such as TB1 (46%), TB7, and TB3 (both 28%). Most live cuttings presented calluses (>70%), characteristic of material of high maturity. Rooting was low for populations (<1.5%) and genotypes (<10%). Branches presented sprouts up to 60 days, with TB presenting the highest total number of sprouts (approximately 300) and the greatest average length (2.8 cm). In general, survival and rooting of cuttings were affected by both populations and genotypes, highlighting TB. A similar response was observed for the branches' sprouting. New studies with more populations, further analyses of the branches' characteristics, and better storage conditions are recommended.

Keywords: Cutting; Detached branches; Erva-mate.

RESGATE E PROPAGAÇÃO VEGETATIVA DE POPULAÇÕES NATIVAS DE Ilex paraguariensis *NO ESTADO DE SANTA CATARINA, BRASIL*

RESUMO – Quando produzidas mudas de ***Ilex paraguariensis*** *através da propagação vegetativa, a seleção correta das populações, genótipos, e ser capaz de obter material juvenil, são fundamentais para seu sucesso. Assim, o objetivo desta pesquisa foi testar sua propagação vegetativa por estaquia e resgate utilizando galhos destacados de diferentes populações do estado de Santa Catarina, Brasil. Desta forma, dois experimentos foram instalados em setembro de 2019 para testar: I) a estaquia de quatro populações, pertencentes aos municípios de Catanduvas (CT), Painel (PL), Três Barras (TB) e Urupema (UR), utilizando dez genótipos escolhidos aleatoriamente de cada, e; II) a brotação epicórmica de galhos destacados destas mesmas populações. Ambos os experimentos foram conduzidos em Lages, Santa Catarina. Em fevereiro de 2020, a estaquia foi avaliada de acordo com as porcentagens de: sobrevivência, calos, enraizamento, novas brotações e permanência das folhas originais. Os galhos foram avaliados a cada 30 dias após seu acondicionamento observando as porcentagens de galhos com brotações, número de brotações e comprimento em centímetros. Para a estaquia, houve baixa*

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sobrevivência em todas as populações (<15%), mas com maior vigor em alguns genótipos, tais como TB1 (46%), TB7 e TB3 (ambos 28%). A maior parte das estacas vivas apresentou calo, característico de material de elevada maturidade. O enraizamento foi baixo nos níveis de população (<1,5%) e genótipo (<10%). Os galhos apresentaram brotações até 60 dias, com TB apresentando o maior número total de brotações (aproximadamente 300) e de maior comprimento médio (2,8 cm). Em termos gerais, a sobrevivência e o enraizamento de estacas foram afetados por ambos população e genótipo, destacando-se TB. Uma resposta similar foi observada para a brotação de galhos. Novos estudos contendo mais populações, análises mais aprofundadas das características dos galhos e melhores condições de acondicionamento são recomendadas..

Palavras-Chave: Estaquia; Galhos destacados; Erva-mate.

1. INTRODUCTION

The species *Ilex paraguariensis* Saint Hilaire (Aquifoliaceae), popularly known as erva-mate or yerba-mate, is widely distributed within the Mixed Rainforest, encompassing Brazil, Argentina, Uruguay, and Paraguay. It is an evergreen and shade-tolerant climax species, which is usually found associated with *Araucaria angustifolia* (Bertol) Kuntze adult individuals, capable of growing and regenerating easily when the shrub and herbaceous strata are thinned (Carvalho, 2003).

In the Brazilian territory, the production of this species is conducted in native areas, enriched or not, or cultivated in plantations, mainly on the state of Paraná and Santa Catarina (Barbosa et al., 2020). It has a wide consumer market, from products that undergo little processing, such as teas from its leaves (Santin et al., 2019), a more intensive processing, such as energy drinks, alcoholic beverages, foods, to its use in the pharmaceutical industry, such as medicines and beauty products, due to its phytochemicals, mainly theobromine and caffeine (Cardozo Junior and Morand, 2016). However, it is a species that can present different amounts of phytochemicals according to the population and genotype (Santin et al., 2017; Vieira et al., 2021), whereas morphological and physiological differences can also be observed (Wendling and Brondani, 2015).

These differences between plants occur due to their sexual propagation, which guarantees a greater genetic variance and range, capable of producing different characteristics within the same species (Xavier et al., 2013). On the other hand, the standardization of its products is affected by that, causing lower productivity and quality, hence, loss of income. Thus, since it is critical that plants be standardized, the correct selection of seedlings is also fundamental, with two options of propagation: resorting to seeds (through sexual propagation) or clones (through vegetative propagation).

The use of *I. paraguariensis* seeds for seedling production can be problematic, in addition to the heterogeneity caused by genetic variation, there are issues regarding emptiness and, for full seeds, physical and morphological dormancies. (Xavier et al., 2013). The morphological dormancy, whereas the embryo is still under development even though being detached from the mother plant, can be considered as the main problem of production of seedlings through seeds, caused by the long periods and variance of germination (Wendling and Brondani, 2015). Consequently, growers and producers must use the available materials, regardless of their genetic quality, hampering standardization, i.e., production and quality.

Focusing on overcoming these challenges, several experiments with this species have been carried out using techniques of vegetative propagation (Wendling et al., 2013; Stuepp et al., 2016, 2017a, 2017b, 2018; Nascimento et al., 2019, 2020, 2022). The main advantage of this technique is the capability to directly select individuals with superior characteristics, enabling the homogenization of plantations and the quality of the final product. Even though there are current studies about this subject, there is a lack of specific vegetative propagation protocols for this species, since a high variation of rooting between individuals is observed (Wendling and Brondani, 2015), which may also be present in same populations (Nascimento et al., 2020, 2022).

Currently, the main challenge to vegetative propagation is to overcome the maturation of *I. paraguariensis* plant material, using techniques that do not require the tree cutting (Wendling et al., 2013; Stuepp et al., 2018), aiming more sustainable procedures that maintain the production. The vegetal materials considered as mature are those that, due to a combination of physiological and ontogenetical characteristics, caused and enhanced according to the increase of age, may lose their totipotency capability and are no longer able to efficiently differentiate its tissues, thus, to produce roots. Furthermore, it is common that the expression of desired plant characteristics to occur only after reaching a certain maturity degree, narrowing the use of its material. Even though, the use of propagules from mature sources can still achieve certain rooting levels, but these are considerably lower in relation to materials of juvenile characteristics (Stuepp et al., 2017a, 2017b; Nascimento et al., 2018).

To this purpose, many rescue techniques can be used to overcome the maturation factor. Essentially, it is necessary to stimulate the development of the dormant buds located in the lower regions of the plant, as this material originated during the first years of plant growth, thus, being ontogenetically younger, i. e., of juvenile characteristics. Among many techniques, the sprouting of physiologically active branches can be also used (Wendling et al., 2013; Nascimento et al., 2018). These branches, after being collected from selected plants, can be stored in a greenhouse under favorable conditions for their sprouting using their energy reserves, working as a continuous source of propagules for further vegetative propagation during its lifespan. Once properly stored, the branches enable the production of juvenile sprouts with higher vigor when compared to those from the canopy, increasing the rooting success (Wendling et al., 2013).

As exposed, two main questions were highlighted in this search. I) Do populations and genotypes influence the vegetative propagation of *I. paraguariensis*? If so, is it possible to use mature material for this purpose? II) Are the *I. paraguariensis* detached branches able to sprout and produce reliable quantities of epicormic sprouts for their vegetative propagation? If so, are they influenced by different populations? For that reason, the objective of this study was to evaluate the vegetative propagation of this species by the technique of cutting considering four different populations and the genotypes within, as well as its vegetative rescue through the sprouting of detached branches considering these same populations.

2. MATERIAL AND METHODS

2.1 Cuttings of different populations and genotypes

Plant material was collected (September 2019) from different regions of Santa Catarina state (SC) on four native production areas (Figure 1), being: Catanduvas (CT) (28°95’67” S; 52°78’22” W), Painel (PL) (27°84’97” S; 49°93’54” W), Três Barras (TB) (26°12’19” S; 50°21’62” W) and Urupema (UR) (27°96’52” S; 49°83’92” W). All locations belong to a *Cfb* climate, with average yearly temperature between 14.1 °C and 17.9 ° C, and with well-distributed yearly rainfall between 1,634 mm and 1,895 mm.

The mother plants were selected according to their sanity and phenotypic similarity, taking into consideration their diameter, height (visually) and proportion of their areal part, totaling ten individuals from each location. The individuals were selected with at least 50 m apart to ensure greater genetic diversity within the same population. As a native area criterion, only shaded mother plants from other higher strata species of plants were chosen, such as from *Araucaria angsutifolia* (Bertol.) Kuntze. Aiming to reduce the effect of physiological age, the mother plants selected from the same population had similar diameters. The averages (and standard error) of diameter obtained were 9.5 (± 0.7) cm for CT; 8.2 (± 0.9) cm for PL; 7.8 (± 0.7) cm for TB; and 6.5 (± 0.6) cm for UR.

From the previously selected mother plants, the canopy sprouts produced in the year were collected, i. e., a high maturity level material. To reduce the influence of ontogenetic age between individuals, the sprouts were collected above the diameter at breast height (DBH – 1.3 m), up to 1.9 m, approximately. The sprouts were obtained with pruning shears and stored in a Styrofoam box with cooled water to avoid excessive dehydration during transportation to the Forest Nursery at the University of Santa Catarina State (UDESC) in Lages (SC) (27°47’33” S; 50°18’04” W). This municipality has *Cfb* climate and an average yearly rainfall of 1,441 mm.

After transportation, the sprouts were cut into cuttings with at least one leaf of reduced area (50%) to avoid excessive transpiration. Rectangular trays (20

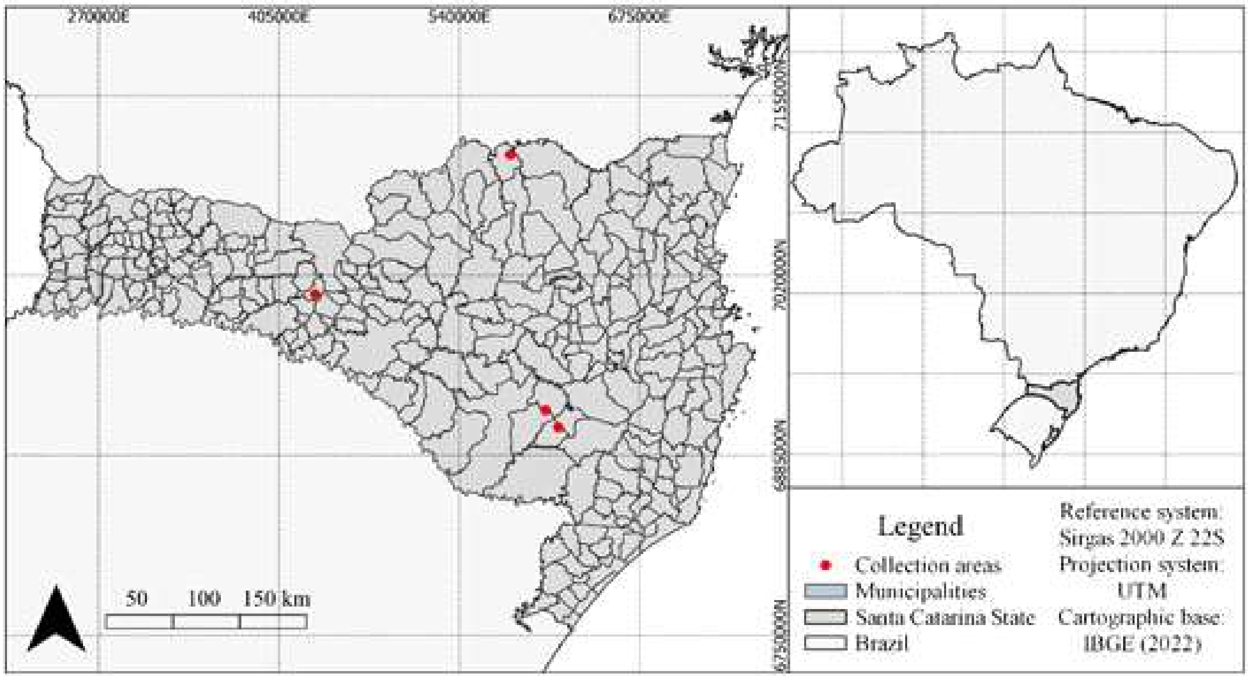

Figure 1 – Collection areas of *I. paraguariensis* vegetal material in the municipalities of Catanduvas (West), Três Barras (North), Urupema (South/bottom) and Painel (South/upper), in Santa Catarina state, Brazil.
Figura 1 *– Áreas de coleta de material vegetal de* ***I. paraguariensis*** *nos municípios de Catanduvas (oeste), Três Barras (norte), Urupema (sul/inferior) e Painel (sul/superior), no estado de Santa Catarina, Brasil.*

L) were used as containers, containing a homogeneous mixture of commercial substrate (mixture of *Pinus* spp. Bark, ashes and peat, pH: 5.4, density: 310 kg m^{-3}) and thin vermiculite (1:1 v/v), with the addition of 6 g L^{-1} of controlled release fertilizer (N[15]-P[9]-K[12]). The purpose of using controlled release fertilizer was only to guarantee the minimum nutritional for rooted cuttings. The trays were filled to approximately 2/3 of their total volume with the prepared substrate mixture, undergoing irrigation (field capacity) to provide support for the cuttings. Each tray was representative of a genotype from a specific population.

The trays containing the cuttings were placed in a mini tunnel system with automatic micro-sprinkler irrigation, composed of four daily irrigations (9:00 AM, 12:00 PM, 3:00 PM and 6:00 PM) of five minutes each, inserted inside a greenhouse with shading (50%). The temperature and relative humidity were measured using a datalogger every ten minutes (Figure 2).

Both genotypes and populations were considered as treatments. At 150 days of implantation of the experiment (February 2020), the cuttings were evaluated in percentages of their survival, rooting, callus formation, presence of new sprouts and permanence of original leaves. Alive cuttings were considered those still presenting green material without any signs of oxidation, rooted or not, with or without leaves and sprouts. Calluses were considered present when alive cuttings had any proportions of an indifferent mass material at their bottom, rooted or not. Rooted cuttings were the ones with well-formed roots, either with high secondary root number or with a root primordium of at least 0.5 cm. New sprouts were considered when one or more new leaves were present, formed after the cutting process. Original leaves were the ones maintained during the cutting process, evaluated according to their permanency or not at the final evaluation.

The experiment was carried out in a completely randomized design, evaluating separately into population and genotypes. The experiment was implemented at the end of September, as there is the beginning of the return of the increase in temperatures (reactivation of vegetative growth) in this period. On average, 50 cuttings were produced for genotype treatments (individuals) and approximately 500 for

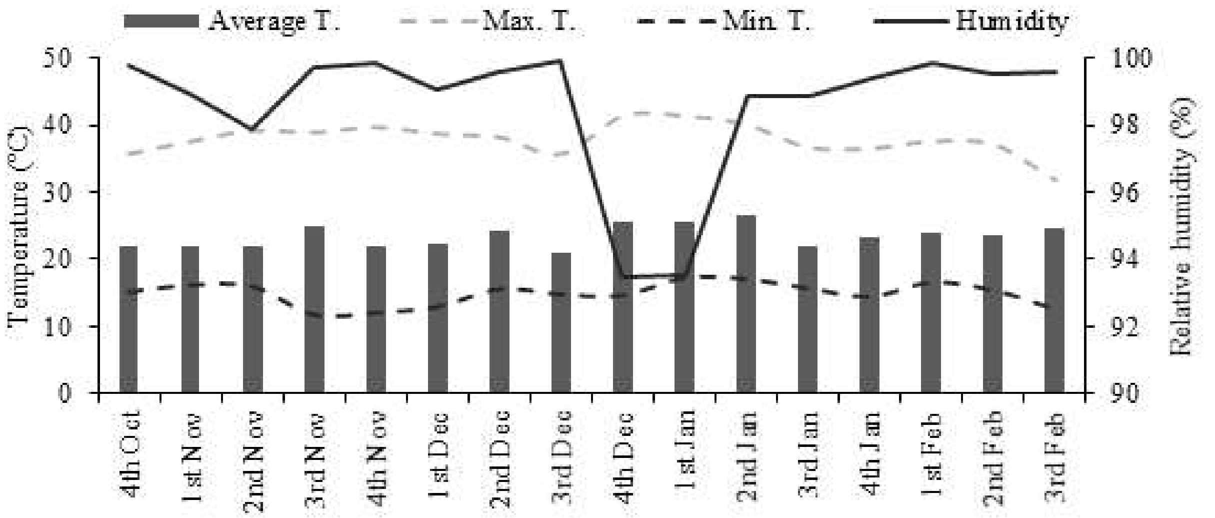

Figure 2 – Average, maximum, minimum temperatures (°C) and relative humidity (%) according to the weeks of *I. paraguariensis* vegetative propagation experiments.
Figura 2– *Temperatura média, máxima, mínima (°C) e umidade relativa (%) de a acordo com as semanas dos experimentos de propagação vegetativa de* ***I. paraguariensis***.

population treatments. Each repetition consisted of ten cuttings, totaling five repetitions per individual.

2.2 Epicormic sprouting from detached branches

Branches from the same previous mother plants were also cut and collected, keeping the same genotypes. They were collected with a manual pruning saw, removing the remaining sprouts and leaves. Longer branches were divided into smaller ones, separated individually, varying between 20 cm and 50 cm long. Morphologically, two types of branches were obtained, but were not considered as treatments in this experiment. The first type being green materials or with some initial degree of lignification, formed from the buds of the year of the mother plants' canopy (tip), with a general average diameter (and standard error) of 13.3 (± 0.2) mm. The second type being a woody, bifurcated material of larger diameters of general average (and standard error) of 32.9 (± 0.4) mm, since they did not present branches with buds of the year at the collection time (mainly the material from TB and UR). Both typologies were collected above DBH.

The ends of the branches were sealed using plastic and tape to prevent excessive dehydration, stored in 50 L trash bags, lightly humidified and sealed. They were transported to the Forest Nursery of the University of the State of Santa Catarina (UDESC) in Lages (SC) and were staked in the vertical position, following their natural disposition, in trays (20 L) containing vermiculite, and were stored under the same conditions as the cutting experiment. Each tray with branches represented one population.

Since the number of branches may not have been statistically adequate to be evaluated at the genotype level, the experiment was carried out considering only the populations. The beginning and finishing of sprouting were evaluated every 30 days, observing the number of branches with new sprouts in percentage, the number of sprouts produced, and the length of sprouts in centimeters. In addition, three categories were determined for possible uses of these sprouts (adapted from Wendling et al., 2013), considering: greater than or equal to 4 cm, which could be used for cutting; between 4 cm and greater than or equal to 2 cm, which could be used for micropropagation, and; less than 2 cm, without immediate use.

The experiment was carried out in a completely randomized design, in a 4x2 factorial scheme, with "A" being the four populations (CT, PL, TB and UR) and "B" the evaluation periods (every 30 days), consisting of two evaluations. Each branch was considered a repetition, totaling ten repetitions for each population.

2.3 Statistical analysis

The data normality through was verified the Shapiro-Wilk test (p<0.05) and transformed by the Box Cox test if necessary. Then, the data was submitted to ANOVA followed by the Scott-Knott average test (p<0.05). The statistics were performed using the R statistical software (R CORE TEAM, 2020).

Aiming a higher reliability to the analyses performed, an Experimental Accuracy (EA%) in percentage was calculated for significant treatments by ANOVA (Equation 1). It evaluates the degree of confidence in the experiment, observing values for selection purposes, considering the ranges: less than or equal to 50% (low); between 50% and 70% (moderate); between 70% and 90% (high), and; greater than 90% (very high) (Navroski et al., 2013). EA% is given by the formula:

$$EA\%=\sqrt{(1-(1/Fcal))}\ \times 100 \quad \text{(Eq. 1)}$$

Whereas: $Fcal = F$ value obtained in the ratio of the mean squares of the treatment (*QMtreat*) and residue (*QMres*), from analysis of variance (ANOVA).

3. RESULTS

3.1 Cuttings of different genotypes and populations

There was no statistical significance for the rooting of cuttings for the populations. For the genotypes, all variables were significant, but only 17 genotypes presented live cuttings at the end of the experiment (Table 1). The results of EA% were considered at least high (>70%) for all variables in both schemes.

The survival of cuttings was below 15% considering the populations and varied between

Table 1 – Percentages of survival, callus formation, rooting, new sprouts, and permanence of original leaves of *I. paraguariensis* cuttings of four native populations of Santa Catarina state, Brazil.
Tabela 1 *– Porcentagens de sobrevivência, formação de calos, enraizamento, novas brotações e permanência das folhas originais de estacas de quatro populações nativas de* ***I. paraguariensis*** *do estado de Santa Catarina, Brasil.*

Population	Survival	Callus formation	Rooting	New sprouts	Permanence of original leaves
	%				
CT	1.4 c*	1.2 c	0.0	1.0 b	0.4 b
PL	2.6 b	2.2 c	1.0	2.2 b	0.4 b
TB1	2.2 a	6.8 a	1.2	8.6 a	2.6 a
UR	3.0 b	3.0 b	0.6	2.0 b	1.4 a
p	<0.0001	<0.0001	0.1190	<0.0001	0.0017
EA%	99.6	96.3	ns	98.7	87.8
Genotypes**	%				
CT 1	2.0 f	0.0 d	0.0 c	2.0 f	0.0 b
CT 5	2.0 f	2.0 d	0.0 c	2.0 f	0.0 b
CT 6	4.0 f	4.0 d	0.0 c	4.0 e	0.0 b
CT 9	2.0 f	2.0 d	0.0 c	0.0 f	2.0 b
CT 10	4.0 f	4.0 d	0.0 c	2.0 f	2.0 b
PL 6	6.0 e	6.0 d	2.0 c	6.0 e	0.0 b
PL 7	20.0 c	16.0 b	8.0 a	16.0 c	4.0 b
TB 1	46.0 a	32.0 a	6.0 a	32.0 a	12.0 a
TB 2	12.0 d	4.0 d	2.0 c	8.0 d	2.0 b
TB 3	28.0 b	20.0 b	4.0 b	18.0 c	8.0 a
TB 6	2.0 f	2.0 d	0.0 c	2.0 f	0.0 b
TB 7	28.0 b	10.0 c	0.0 c	26.0 b	4.0 b
TB 8	6.0 e	0.0 d	0.0 c	2.0 f	0.0 b
UR 5	2.0 f	2.0 d	0.0 c	0.0 f	0.0 b
UR 8	4.0 f	4.0 d	0.0 c	0.0 f	2.0 b
UR 9	12.0 d	12.0 c	2.0 c	10.0 d	8.0 a
UR 10	12.0 d	12.0 c	4.0 b	8.0 d	4.0 b
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
EA%	99.2	96.2	75.2	97.4	86.5

CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Experimental Accuracy in percentage; ns: not significant; *Averages followed by the same letter do not differ from each other according to the Scott-Knott average test (p<0.05); **Only the surviving genotypes are present, 17 out of 40.
*CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Acurácia Experimental em porcentagem; ns: não significativo; *Médias seguidas de mesma letra não se diferem de acordo com o teste de médias Scott-Knott (p<0,05); **Somente os genótipos sobreviventes estão presentes, 17 de 40.*

46% and 2% for the surviving genotypes. The TB population presented the highest survival percentage, at least four times higher than the others. This population presented six genotypes alive, highlighting TB1, TB3 and TB7, all of them with at least 25% of survival. The populations of CT had the lowest survival average (five genotypes alive), followed by PL (two genotypes) and UR (four genotypes).

Callus formation was similar to the survival percentages of the CT, PL and UR populations, which indicates that most of the alive cuttings presented calluses (less than 0.4% of difference between variables). This same relation was observed at the genotype level in all populations. On the other hand, the population of TB did not follow this perspective, with approximately 50% of cuttings with calluses.

Rooting did not reach 2% and 10% for the population and genotypes, respectively. The CT population did not obtain any rooted cuttings, while the populations of UR, PL and TB presented 0.6%, 1.0% and 1.2%, respectively, without statistically significance. For the genotypes, only seven (out of 40) presented at least one rooted cutting, highlighting the genotypes of PL7 and TB1, with more than 5% rooting.

Regarding the presence of new sprouts and permanence of original leaves, both presented a similar response to the callus formation. For the populations of CT, UR and PL, almost every cutting alive presented new sprouts and leaves, while TB presented a percentage nearly two-thirds of its survival percentage. Furthermore, the TB population and its genotypes also presented low results of original leaves permanency when compared to survival.

3.2 Epicormic sprouting from detached branches

Epicormic sprouting started approximately 20 days after the branches were staked in the mini tunnel system. However, only two evaluations (30 and 60 days) occurred, as sprouting and former sprouts were no longer detected on further evaluations.

A significant factorial interaction between the populations and the evaluation periods for sprouting and number of sprouts was detected, while the interaction for the length of sprouts was not detected (Table 2). For this variable, only the populations were statistically different ($p<0.0001$; EA%=97.1) while the evaluations were not ($p=0.5198$; EA%=ns).

The first evaluation provided the best results for branches with sprouts as well as for number of sprouts, with TB and UR producing more than 25 sprouts on average, while other populations presented half as many sprouts for this period. However, there was a drastic decrease in sprouting and on the average number of sprouts during the second evaluation, mainly for UR, which had the greatest decrease among all populations.

Table 2 – Percentage of branches with new sprouts, number of sprouts and length of sprouts in centimeters of *I. paraguariensis* detached branches of four native populations of Santa Catarina state, Brazil.
***Tabela 2** – Porcentagem de galhos com novas brotações, número de brotações e comprimento de brotações em centímetros de galhos destacados de quatro populações nativas de **I. paraguariensis** do estado de Santa Catarina, Brasil.*

Evaluation	Population				
	CT	PL	TB	UR	Average
Branches with new sprouts (%) (p=0.0004; EA%=91.9)					
30 days	100.0 aA*	100.0 aA	90.0 aA	100.0 aA	98.0
60 days	20.0 bB	20.0 bB	70.0 aA	10.0 bB	34.0
Average	60.0	60.0	80.0	55.0	66.0
Number of sprouts (p=0.0006; EA%=91.6)					
30 days	12.8 bA	12.8 bA	28.8 aA	26.9 aA	19.4
60 days	0.7 bB	0.6 bB	7.5 aB	0.1 bB	2.4
Average	6.8	6.7	18.2	13.5	10.9
Length of sprouts (cm) (p=0.6523; EA%=ns)					
30 days	1.0	0.9	2.8	0.7	1.2
60 days	0.6	0.9	3.0	0.1	1.1
Average	0.8 b	0.9 b	2.9 a	0.4 b	1.2

CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Experimental Accuracy in percentage; ns: not significant; *Averages followed by the same letter do not differ from each other according to the Scott-Knott average test ($p<0.05$).
CT: Catanduvas; PL: Painel; TB: Três Barras; UR: Urupema; EA%: Acurácia Experimental em porcentagem; ns: não significativo; *Médias seguidas de mesma letra não se diferem de acordo com o teste de médias Scott-Knott ($p<0,05$).

As for length, the TB population presented sprouts at least three times longer than all other populations. The CT and PL populations presented sprouts of nearly 1 cm during both evaluations. Meanwhile, UR had the shortest sprouts, meaning that this population had a high number of sprouts of short length during the first evaluation.

Regarding the categorization the epicormic sprouts according to their length, these presented considerable differences between the evaluation periods of the present study (Figure 3). During the first evaluation, only TB presented a higher quantity of sprouts longer than 4 cm as well as between 2 cm and 4 cm (both approximately 35 sprouts). For the second evaluation, even though the branches of all populations presented a considerable decrease in the sprouts number, the ones that remained continued their development. In this scenario, TB had an increase of the quantity of sprouts longer than 4 cm (approximately 57).

4. DISCUSSION

4.1 Cuttings from different genotypes and populations

EA% was considered at least high (>70%) for both populations and genotypes. EA% analysis aims to asses, alternatively, the degree of confidence in the experiment, in the performed analyses and in the statistics applied. It may also be used as a criterion for further selection of among different materials (Navroski et al., 2013). Nascimento et al., (2022) highlights elevated EA% (>90%) values for the same variables tested in the present search, in two populations of the same species. As well as in the present search, the authors also observed a high EA% (>80%) for their genotypes, thus indicating that the conducted analyses were carried out with precision and reliability.

Even though the plant material of *I. paraguariensis* was collected at the best time for its survival and rooting according to literature, which is spring (Stuepp et al., 2017a; Sá et al., 2018), they presented little adaptation to the new climate conditions. This was possibly affected by the unfavorable conditions during the experiment found inside the mini tunnel (Figure 1). Although humidity (>90%) was ideal for the maintenance of propagules (Xavier et al., 2013), the excessive temperatures during daytime (sometimes above 40 °C) and the thermal amplitude between day and night (up to 25 °C) were possibly the main reason for the low survival and further results.

Even though rooting environments of low technological investments may not present significant

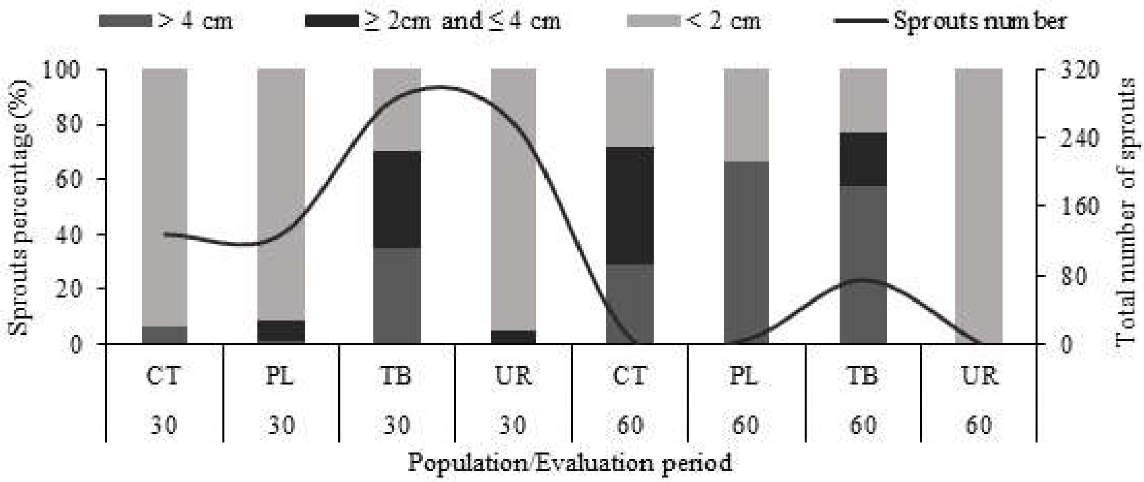

Figure 3 – Total number and percentage of sprouts in three categories of *I. paraguariensis* detached branches of four native populations of Santa Catarina state, Brazil, in two evaluations periods; CT: Catanduvas, SC; PL: Painel, SC; TB: Três Barras, SC; UR: Urupema, SC.

Figura 3 *– Número total e porcentagem de brotações em três categorias de galhos destacados de quatro populações nativas de* ***I. paraguariensis*** *do estado de Santa Catarina, Brasil, em dois períodos de avaliação; CT: Catanduvas, SC; PL: Painel, SC; TB: Três Barras, SC; UR: Urupema, SC.*

differences in the maintenance of *I. paraguariensis* cuttings from the ones with more technological advancements, they are more susceptible to external environmental variations, mainly temperature (Stuepp et al., 2017a). According to Nascimento et al., (2020), when submitted to two different rooting environments, one with temperature control (adapted for *Eucalyptus* sp. cutting) and the other being a simplified structure, *I. paraguariensis* cuttings presented a higher survival percentage inside the non-controlled temperature environment (approximately 20% more). It is still unknown for completely the survival behavior this species' cuttings regarding temperature variance, besides the necessity of a low variance, which was not present in this experiment.

This variation in survival can also be a direct reflection of the plant material origin, easily observed between and within the populations of the study. It is possible that origin places with lower annual temperatures and lower solar incidence, such as PL and UR, produce materials with greater sensitivity to environmental factors of the rooting environment. The genetics of the mother plant seems to directly influence the survival of propagules (Vieira et al., 2021), but it can be favored or affected according to the climatic conditions of the mini tunnel. Corroborating with this perspective, Nascimento et al., (2022) observed that the survival of a genotype can vary up to 15% and 20% for UR and TB populations, respectively, according to the rooting environment, while the differences considering genotypes may be higher, up to 50% between the most and least adapted.

For rooting, it is possible that the high degree of maturation of the propagules used, which was observed through the presence of calluses on cuttings, may have been the main factor for the low rooting. The ontogenetic age is a crucial factor for the rooting success of propagules, as a reinvigorated material present a higher possibility due to its higher vigor (Stuepp et al., 2018). Since no reinvigoration techniques were used for the rescue of vegetative material, it is possible that the sprouts collected from the canopy used for cutting presented a higher maturation degree. Cuttings of *I. paraguariensis* tend to have their rooting directly, hardly occurring after the formation of calluses (Stuepp et al., 2017a), and this factor can be easily observed in the present search. However, the low rooting came directly from cuttings that had calluses, indicating that, even though it is initially an undifferentiated tissue, it can still produce roots if remained for a longer period inside the rooting environment (Stuepp et al., 2017b).

Even though the hindering environmental conditions of the present work as well as the use of mature material, some genotypes still presented rooted cuttings, mainly PL7, TB1 and TB3. Recent searches (Nascimento et al., 2020, 2022; Vieira et al., 2021) show a great variance of rooting from individuals of the same population and established clones. This relation is easily seen in the present study, with a great variability of rooting results from individuals in the same populations. When the containers used for the cutting of *I. paraguariensis* is considered, there is the formation of longer and adventitious roots if the material has more contact with soil, especially when using 1.5 L and 3 L containers (Pimentel et al., 2017). Even though 20 L trays were used as containers in the present search, rooting and root development was minimal.

The presence of leaves in cuttings is crucial for their rooting, since these organs are responsible for the production of auxins (Taiz et al., 2015). In general, there was a considerable sprouting of the surviving cuttings from all populations and genotypes. It is possible that the production of new sprouts and leaves are high dependent on the genotype used, being also favored or affected by the rooting environment.

However, the same did not happen for the original leaves, as most of the cuttings did not present them in the final evaluation, which may also justify the low rooting. The loss of the original leaves and the production of new sprouts may be a response of an excessive stress undergone by the cuttings, using their remnant energy on new aerial organs. The collection of *I. paraguariensis* vegetative material for propagation during spring can maintain more than 75% of cuttings with leaves, considering a rooting environment with irrigation though nebulization (Sá et al., 2018). Therefore, the leaf fall in the present study may be due to the high stress conditions of the rooting environment, as previously cited, being increased or decreased according to the genotype tested.

4.2 Epicormic sprouting from detached branches

Even though some factors were not considered when this experiment was carried out, I. e., the two branches' typologies, their ontogenetic age and the storing position of the branches (vertical or horizontal), it is important to cite the possible consequences of these post assessed conditions.

The two types of branches, which had singular shapes and diameters, may have caused the differences of sprouting and number of sprouts observed (Nascimento et al., 2018). The thicker and more lignified branches were of larger diameters (, what possibly allowed a greater number of buds to be stored during their development, enabling higher sprouting percentages and number of sprouts for the populations of TB and UR. Materials with good surface areas have greater chances to sprout and in higher quantities, as they develop at a slow pace, thus storing more dormant buds (Stuepp et al., 2016).

The ontogenetic age may have affected the development of the branches as well, as canopy material may have had lower vegetative vigor than those obtained in the lower parts of the mother plants. This might be true between the two typologies, but not between populations, as branches from similar heights of the mother plants were obtained between TB and UR, which diverged on the results. Therefore, it is possible that the determining factor was the populations themselves, resulting in a higher/lower vigor and amounts of energy reserves for those materials. Furthermore, the storing environment also had a crucial role in the development of the sprouts, as this material may have oxidized faster for UR, a material of higher sensitivity. when compared to TB, due to the higher temperatures of the mini tunnel system (Xavier et al., 2013).

The branches' storing position can affect their useful life, in which Nascimento et al., (2018) tested the epicormic sprouting of *I. paraguariensis*' branches stored horizontally, observing their development up to 300 days. However, Wendling et al., (2013) determined that vertically stored sprouts can produce good quality sprouts up to 70 days, being a similar result to the present search. The relation between the branches' diameter and storing position could allow new interpretations and results, being an interesting subject to be tested in this species in new experiments.

Regarding the classification of the sprouts according to their length, it is possible to notice in the initial evaluation a greater number of sprouts shorter than 4 cm without an immediate use for cutting, similar to the results obtained by Wendling et al., (2013). However, sprouts between 2 cm and 4 cm could still be used for micropropagation, allowing an alternative way for the vegetative propagation of the species. Since plant improvement is a not easy task for *I. paraguariensis* due to its problems with conventional methods of selection by seeds (Wendling and Brondani, 2015), the vegetative rescue of this species is fundamental for this purpose. Therefore, as branches are easy to obtain and to produce sprouts, evaluating the sprouting variables in these materials may prove essential to the formulation of new vegetative propagation protocols.

5. CONCLUSION

The first hypothesis of this search was confirmed. A possible superiority was detected according to certain populations and genotypes for vegetative propagation of *I. paraguariensis*, highlighting Três Barras (TB). However, it is possible that the association between material maturity and excess of temperature hampered the survival of cuttings from all origins.

Both conditions of the second hypothesis of this search were confirmed. *I. paraguariensis*' detached branches produced different quantities of sprouts according to the populations. On the other hand, they were also probably affected by the different branch typologies, the harsh conditions of the storage environment, and the vertical/horizontal storage sense, which were not considered in this search.

Thus, it is recommended: I) New studies with different populations and genotypes for the vegetative propagation through cutting, testing materials of juvenile characteristics to confirm with certainty any superiorities; II) New studies with different populations for the vegetative rescue through detached branches, considering the typologies and the two storing senses. Furthermore, both recommendations can be followed by better environmental conditions for material storing.

AUTHOR CONTRIBUTIONS

Conceptualization: Nascimento B, Navroski MC, Pereira, MO, Mantovani, A. Performed the analyses: Nascimento B, Sá ACS, Corrêa BJS, Schilisting T. Analysis of results: Nascimento B, Navroski MC, Mantovani A. Statistical analysis: Nascimento B, Navroski MC. Writing-original draft: Nascimento B. Writing-review and editing: Nascimento B. Supervision and coordination of research: Navroski MC, Mantovani A.

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