

Epicormic sprouting from detached branches of *Ilex paraguariensis* A. St.-Hil.: effects of disposition sense and branch diameter on propagation potential

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ABSTRACT

Background: Epicormic sprouting represents a promising strategy for vegetative rescue and propagation of mature trees; however, factors influencing sprout productivity and viability remain insufficiently understood for *Ilex paraguariensis*. This study evaluated the epicormic sprouting potential of detached branches according to disposition sense (horizontal and vertical) and branch diameter (thin and thick), as well as the adventitious rooting performance of cuttings derived from epicormic and canopy sprouts. Detached branches were maintained under controlled mini-tunnel conditions and assessed over a 120-day period for survival, sprouting percentage, number of sprouts and sprout length. Subsequently, cuttings from both material sources were evaluated for survival, callus formation, rooting and sprout development.

Results: Disposition sense was the main factor influencing sprouting dynamics. Vertically disposed branches exhibited faster initial sprouting but reduced longevity, whereas horizontally disposed branches showed slower development combined with extended survival. Branch diameter had secondary effects, mainly influencing early sprouting responses. Epicormic cuttings presented lower survival compared to canopy-derived material; however, rooting percentages were statistically similar between sources.

Conclusions: These findings demonstrate that detached branches constitute a viable *ex situ* source of epicormic sprouts, allowing flexible management strategies depending on production goals. Nevertheless, improvements in environmental conditions and propagule physiological status may be required to enhance survival in vegetative propagation. Overall, detached branches represent a promising alternative for the propagation and conservation of *I. paraguariensis*, supporting clonal propagation programs while minimizing damage to the selected trees.

Keywords: Cuttings; mate; ontogenetic age; vegetative propagation.

HIGHLIGHTS

Branch disposition was the main factor controlling epicormic sprouting dynamics
Vertical branches promoted faster sprouting but showed reduced longevity
Horizontal branches ensured longer survival and sustained sprout production
Epicormic sprouts showed rooting performance comparable to canopy material.

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INTRODUCTION

Ilex paraguariensis A. St.-Hil. (Aquifoliaceae), commonly known as yerba-mate, is a native tree species widely distributed in southern South America, including Brazil, Paraguay, Argentina and Uruguay (Carvalho, 2003). The species has significant economic importance due to the presence of bioactive compounds such as caffeine and theobromine, which support its use in food, cosmetic and pharmaceutical industries (Santin *et al.*, 2019). In southern Brazil, *I. paraguariensis* production represents an important source of income, especially for smallholder systems, contributing substantially to regional economies (IBGE, 2024).

Despite its economic relevance, maintaining productive plantations can be challenging. Successive harvesting cycles and physiological aging often lead to productivity decline, requiring replacement or the reinvigoration of former individuals. Seed-based propagation presents additional limitations, since *I. paraguariensis* seeds frequently exhibit multiple dormancy mechanisms, particularly morphological dormancy, which can significantly delay germination and hinder the large-scale production of uniform planting material (Duarte *et al.*, 2025). Consequently, vegetative propagation has been widely investigated as an alternative strategy to ensure clonal uniformity and enable genetic improvement programs (Xavier *et al.*, 2013; Wendling and Brondani, 2015).

The success of vegetative propagation in woody species is strongly influenced by ontogenetic age and physiological status of the propagules. Juvenile tissues generally exhibit higher morphogenic competence, reflecting greater cellular totipotency and rooting capacity (Wendling *et al.*, 2014a; Hartmann *et al.*, 2018). In mature trees, dormant buds preserved along the stem may retain characteristics associated with earlier developmental stages, and their activation through vegetative rescue techniques can partially restore morphophysiological competence, acting as a reinvigoration process rather than true rejuvenation. In woody species, rejuvenation implies a complete reversal to the juvenile phase, whereas reinvigoration corresponds to the temporary recovery of physiological vigor without altering the ontogenetic phase (Wendling *et al.*, 2014a; Wendling *et al.*, 2014b).

In this context, vegetative rescue techniques have been widely described as promoting rejuvenation and/or reinvigoration effects, depending on the species and propagation conditions (Stuepp *et al.*, 2018). Methods such as girdling or coppicing are commonly employed to induce epicormic sprouting by altering hormonal balance and promoting cytokinin accumulation; however, these practices may compromise tree health or increase management risks when applied intensively (Stuepp *et al.*, 2017; Nascimento *et al.*, 2018).

An alternative approach involves the use of detached branches as an *ex situ* source of epicormic sprouts. Recent studies have demonstrated the efficiency of epicormic forcing techniques for woody species under controlled conditions, enabling the production of physiologically active sprouts with increased morphogenic potential,

although not necessarily representing true juvenile tissues, since their development remains influenced by the ontogenetic gradient of the donor plant (Wendling *et al.*, 2014b; Stuepp *et al.*, 2017; Maqbool and Aftab, 2024; Schatz Sá *et al.*, 2024).

In this context, physiologically active branches collected from selected genotypes can produce sprouts from dormant buds when maintained under adequate environmental conditions, allowing the production of propagules without severe damage to selected trees (Wendling *et al.*, 2013; Nascimento *et al.*, 2018; Lima *et al.*, 2020; Silva *et al.*, 2021a; Silva *et al.*, 2021b; Nascimento *et al.*, 2023; Schatz Sá *et al.*, 2024; Duarte *et al.*, 2025). This technique enables the continuous availability of material for vegetative propagation methods such as cutting or micropropagation (Wendling and Brondani, 2015). However, detached branches remain subject to ontogenetic constraints, and factors such as development medium, season of the year, branch position, morphology and physiological condition may influence sprouting performance and longevity (Xavier *et al.*, 2013; Maqbool and Aftab, 2024).

Among the variables potentially affecting epicormic sprouting, branch diameter and disposition during storage have received limited attention. Previous studies indicate that these factors may alter resource allocation, hormonal distribution and hydraulic functioning, potentially influencing sprout productivity and survival, but their combined effects remain insufficiently explored for *I. paraguariensis* (Nascimento *et al.*, 2018; Nascimento *et al.*, 2023). Furthermore, although epicormic sprouts are often considered promising propagules for this species due to their reinvigorated characteristics, their effectiveness for rooting compared to mature canopy material requires further evaluation.

Previous studies have demonstrated that the rooting capacity of cuttings is strongly influenced by ontogenetic age, stock plant management and propagation conditions, reinforcing the importance of properly understanding the physiological status of propagules used in vegetative rescue strategies (Wendling *et al.*, 2005; Wendling and Brondani, 2015; Stuepp *et al.*, 2017). Additionally, clonal propagation techniques such as mini-cutting have shown superior performance compared to seed-derived plants in terms of growth and productivity, highlighting the importance of vegetative propagation for the establishment of more uniform and productive plantations (Wendling and Brondani, 2015).

Based on these considerations, the present study tested the following hypotheses: (i) detached branches represent an effective source of epicormic sprouts for *I. paraguariensis*; (ii) sprouting dynamics are influenced by branch diameter and disposition sense during storage; and (iii) epicormic sprouts derived from detached branches present rooting performance comparable to canopy-derived material. Therefore, the objective of this study was to assess the epicormic sprouting potential of detached branches according to diameter and disposition sense, as well as to evaluate the rooting performance of cuttings produced from this source in comparison with mature canopy material.

MATERIAL AND METHODS

Sprouting of the detached branches

Detached branches were collected from mature *I. paraguariensis* trees growing in a native population located in a Mixed Ombrophilous Forest (26°12'19" S; 50°21'62" W), in Três Barras, Santa Catarina State, Brazil, at approximately 762 m above sea level. The region presents a Cfb climate, with an average annual temperature of 17.9 °C and annual rainfall of approximately 1,673 mm.

Selected trees were chosen based on the following criteria: (i) individuals growing under natural shading conditions, commonly beneath *Araucaria angustifolia* or other taller species; (ii) minimum spacing of 30 m between selected trees to increase the probability of genetic variability; and (iii) absence of visible phytosanitary problems or severe defoliation symptoms. A total of ten trees were selected and up to four branches were collected per tree using a pruning saw.

Branches were harvested between 1.0 and 1.5 m above ground level in order to reduce ontogenetic variation associated with height. Secondary or poorly lignified branches were excluded. After collection, leaves, lateral sprouts and secondary branches were removed, and the material was transported in insulated boxes containing cooled water to minimize dehydration.

At the Forest Nursery of Santa Catarina State University (27°47'33" S; 50°18'04" W), in Lages, Santa Catarina, branches shorter than 20 cm or with diameters smaller than 10 mm were discarded to standardize the material. Remaining branches were washed and immersed

for 30 seconds in a decontaminant solution (10 mL L⁻¹) containing Cu (7%), Ca (3%) and S (3%). Branches were then classified into two diameter categories: thin (average: 13.9, standard error: ± 0.1 mm) and thick (average: 30.9, standard error: ± 0.2 mm). Only straight, woody, non-bifurcated branches were used.

Branches were sealed with plastic to reduce water loss, and were arranged under two disposition treatments: vertical position: branches inserted into trays containing vermiculite; horizontal position: branches placed on tube trays without direct substrate contact.

All material was maintained in a mini-tunnel system installed inside a shade house, with temperatures ranging approximately between 15 °C and 35 °C (Figure 1). Irrigation was provided through an automated micro-sprinkler system four times daily (09:00 AM, 12:00 PM, 03:00 PM and 06:00 PM), each event lasting 10 minutes.

Evaluations were performed every 15 days for 120 days (eight assessments), recording: branch survival (%), sprouting (%), average number of sprouts per branch, average sprout length (cm).

Branches were considered alive when presenting hydrated tissues and viable buds or developing sprouts. Sprouting was defined when sprouts reached at least 0.5 cm in length with visible leaf development. Sprout length was measured from the insertion point to the last leaf.

The experiment followed a completely randomized design in a factorial arrangement (2 diameters x 2 disposition senses), with three replicates per treatment, each replicate consisting of five branches. For the branches, specific acronyms were used aiming to clearer and easier to understand text, being: I) Thin branches horizontally disposed – TNH; II) Thick branches horizontally disposed – TKH; III) Thin branches vertically disposed – TNV; IV) Thick branches vertically disposed – TKV.

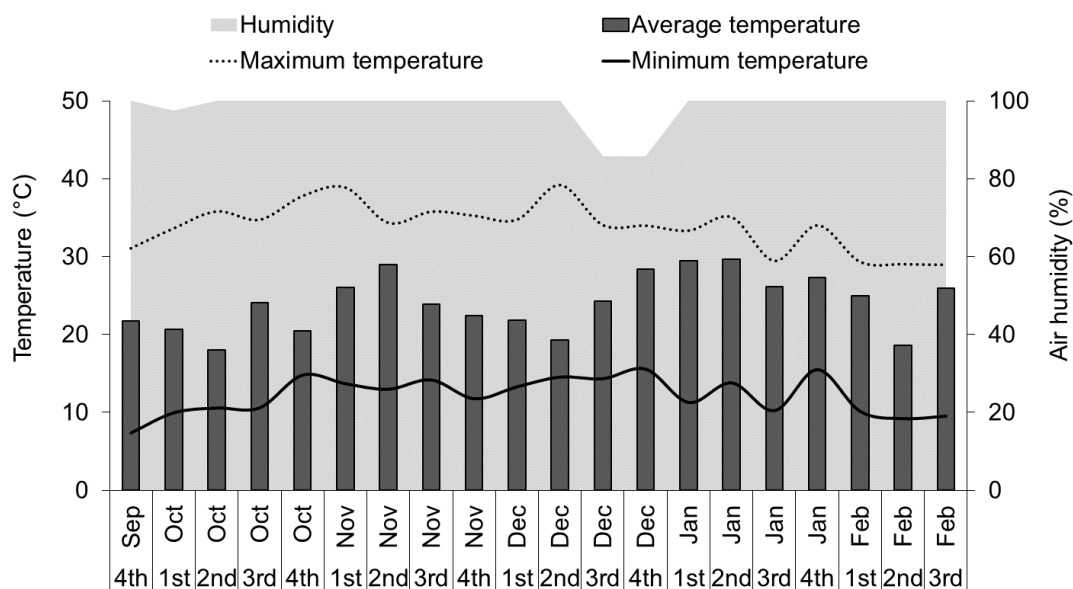


Figure 1: Average, maximum and minimum temperatures (°C), and relative air humidity (%) of the storage environment during the experiment of *Ilex paraguariensis* detached branches sprouting.

Adventitious rooting of canopy and epicormic cuttings

120 days after installation, canopy sprouts were collected from the same population to compare rooting performance between mature canopy material and epicormic sprouts obtained from detached branches.

Collection procedures followed the same selection criteria described previously. Canopy sprouts were harvested from heights above 2 m using pruning shears. Both canopy and epicormic sprouts were prepared as cuttings approximately 7 cm long, each containing at least one leaf reduced to 50% of its original area to minimize transpiration. All material was temporarily stored in fresh water prior to planting. No growth regulators were applied in this experiment.

Cuttings were inserted into 110 cm³ tubes filled with commercial substrate composed of peat, vermiculite, class "A" organic waste and limestone (pH 5.5; density 130 kg m⁻³), supplemented with controlled-release fertilizer (15-9-12 NPK) at 6 g L⁻¹. Trays were maintained under the same mini-tunnel environmental conditions described above.

After 120 days, the following variables were evaluated: survival (%), callus formation (%), rooting (%), presence of new sprouts (%), retention of original leaves (%).

Cuttings were considered rooted when presenting root primordia ≥ 5 mm. The experiment followed a completely randomized design with two treatments (canopy VS epicormic origin), eight replicates of twelve cuttings each (total of 192 cuttings).

Statistical analysis

Data were subjected to preliminary tests to verify assumptions of normality (Shapiro–Wilk test; $p > 0.05$) and homogeneity of variances (Bartlett test; $p > 0.05$). When necessary, Box-Cox transformations were applied.

Analysis of variance (ANOVA; $p < 0.05$) was performed for all variables. Temporal responses of the detached branch variables were evaluated through regression analysis. The model used was quadratic, better explaining biological factors, followed by a reliable adjusted R². The regression model was fitted according to the following equation:

$$y = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon$$

Where: y represents the response variable, x the evaluation time, β_0 the intercept, β_1 and β_2 the linear and quadratic coefficients, respectively, and ε the random error term.

Boxplots graphics were applied to better observe sprout development. Pearson's correlation analysis was used to evaluate relationships among variables and branch diameter. For rooting experiments, Student's t -test for independent samples were applied ($p < 0.05$). All analyses were performed using R statistical software (R Core Team, 2024).

RESULTS

Temporal dynamics of epicormic sprouting

Epicormic sprouting of detached branches was first detected at 30 days after installation. Before this period, dormant buds exhibited initial development but were not considered sprouts due to lengths shorter than 0.5 cm or absence of developed leaves.

Across treatments, sprouting responses varied over time, showing an initial increase followed by a progressive decline in productivity. This is observed in survival (Figures 2A and 2B), sprouting (2C and 2D), number of sprouts (2E and 2F) and partially for length of sprouts (2G and 2H). Regression analyses indicated distinct temporal patterns depending on disposition sense and branch diameter.

Horizontally disposed branches (TNH and TKH) maintained high survival rates until approximately 90 days, followed by a reduction of nearly 50% toward the final evaluation. In contrast, vertically disposed branches (TNV and TKV) showed faster declines in survival, with no living branches remaining after 75 days (Figures 2A and 2B).

Sprouting percentages followed similar trends (Figures 2C and 2D). TNH exhibited early sprouting responses, reaching approximately 30% at 30 days and a maximum of 50% at 60 days, followed by gradual decreases. TKH presented slower initial sprouting but maintained relatively stable sprouting levels after 45 days. Among vertical treatments, TNV reached nearly 50% sprouting during early evaluations and stabilized around 61% before declining, whereas TKV increased from approximately 11% at the first evaluation to a maximum of 67% at intermediate assessments, followed by reductions after 60 days.

The number of sprouts was more affected by the disposition sense rather than the branch diameter (Figures 2E and 2F). Vertically disposed branches produced a maximum of 4.4 and 4.5 sprouts at the second and third evaluations for thin and thick branches, respectively. Horizontally disposed branches achieved maximum sprout number at 45 days, with averages of 6.2 and 2.1 for thick and thin branches, respectively.

Sprout elongation differed according to both disposition sense and branch diameter (Figures 2G and 2H). TNH produced longer sprouts during early evaluations, reaching average lengths of approximately 0.8 cm by 60 days. Conversely, TKH showed increased elongation during later evaluations, achieving maximum average lengths of 1.6 cm. A similar pattern was observed for vertically disposed branches. TNV initially produced longer sprouts; however, TKV presented greater elongation during subsequent evaluations, reaching average lengths around 0.7 cm.

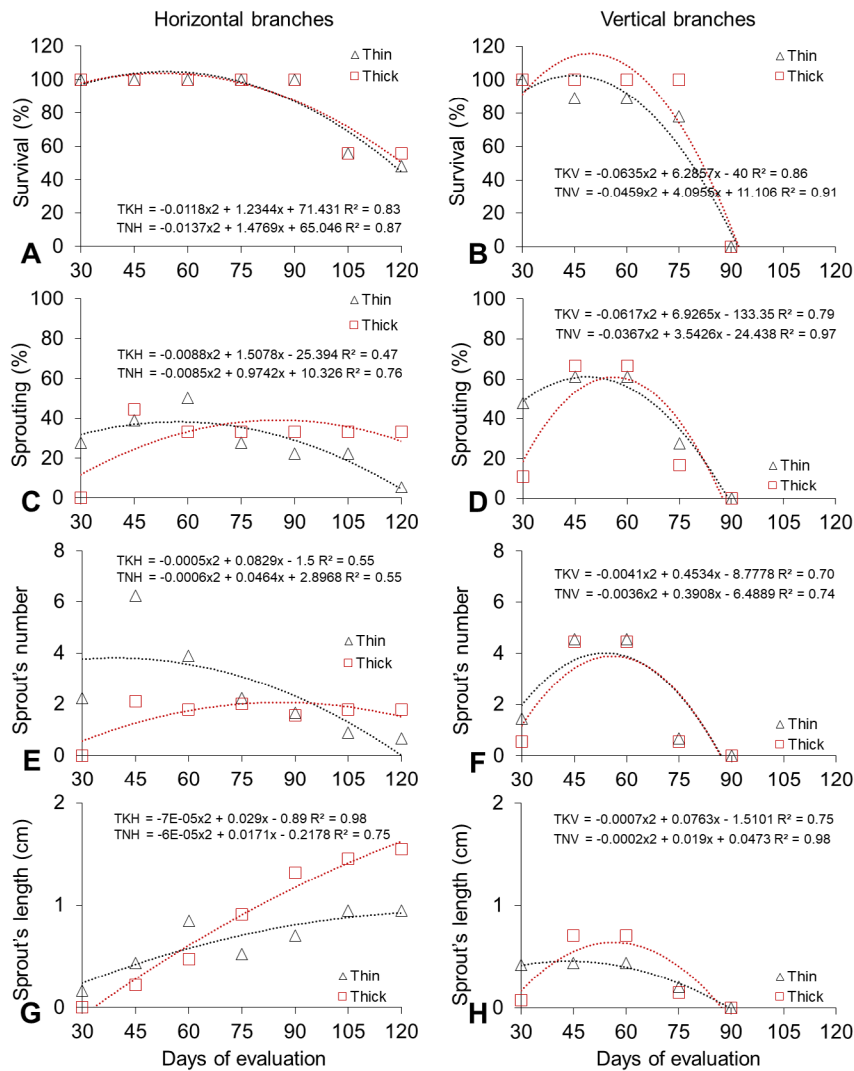


Figure 2: Sprouting dynamics of detached branches of *Ilex paraguariensis* according to branch disposition and diameter. TKH: Thick horizontally disposed branches; TNH: Thin horizontally disposed branches; TKV: Thick vertically disposed branches; TNV: Thin vertically disposed branches.

Treatment effects on sprouting productivity

Overall, a high proportion of branches did not produce sprouts throughout the experiment (Figure 3A). In this aspect, a consistent percentage of sprouting is shown for horizontally disposed branches, differently for the vertical ones, highlighting TKV. On average, nearly 50% of the sprouts belonging to TKH were longer than 0.5 cm, with values reducing proportionally for each other category of diameter and disposition sense (Figure 3B). However, a great variation of results among evaluations can be noted, especially for TKH.

Thin branches were responsible for producing the highest quantities of sprouts for each diameter condition, however, of short length (Figure 4A). For sprouts longer than 0.5 cm (Figure 4B), the thick branches showed a more consistent production, mainly for TKH. Sprout's length varied greatly according to their disposition rather

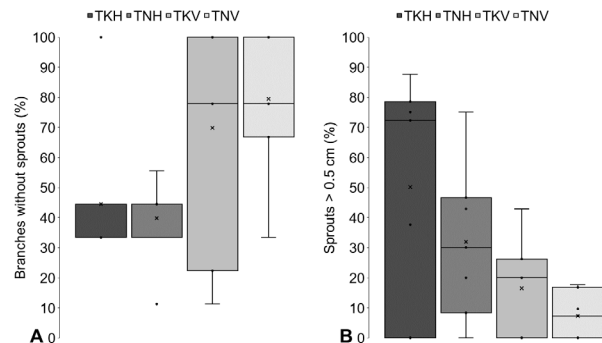


Figure 3: Percentages of number of sprouts (A) and sprouts longer than 0.5 cm (B) of *Ilex paraguariensis* detached branches according to their thickness and disposition sense. TKH: Thick horizontally disposed branches; TNH: Thin horizontally disposed branches; TKV: Thick vertically disposed branches; TNV: Thin vertically disposed branches.

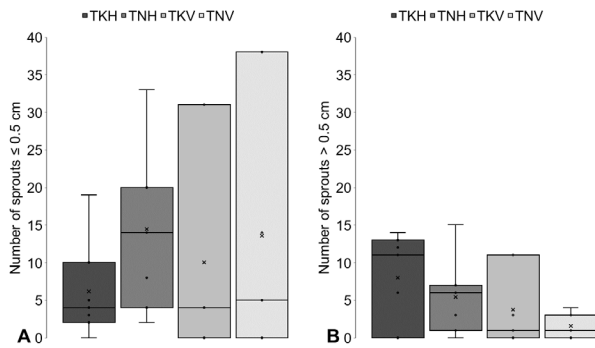


Figure 4: Overview data of number of sprouts according to their length, shorter or equal to 0.5 cm (A) and longer than 0.5 cm (B), of sprouts and their categorization of *Ilex paraguariensis* detached branches according to their thickness and disposition sense. TKH: Thick horizontally disposed branches; TNH: Thin horizontally disposed branches; TKV: Thick vertically disposed branches; TNV: Thin vertically disposed branches.

than thickness, whereas horizontal branches produced materials almost four times longer in average.

Correlation among sprouting variables

Pearson's correlation analysis revealed a very strong correlation between sprouting percentage and sprout length in horizontally disposed branches ($r = 0.93$) (Table 1). Strong correlations were also observed between sprouting and both number and length of sprouts in vertically disposed branches ($r = 0.74$ and $r = 0.75$, respectively). Moderate correlations were detected between branch diameter and survival ($r = 0.58$) and between number and length of sprouts ($r = 0.54$) in vertical treatments, whereas other relationships were weak or negligible.

Table 1: Pearson's correlation of variables related to the sprouting of *Ilex paraguariensis* detached branches, according to their disposition sense, being horizontal or vertical.

Variables		Diameter of branches		Survival of branches		Sprouting of branches		Number of sprouts		Length of sprouts		
Diam. of branches		X										
Surviv. of branches		0.00*	0.58	X								
Sprout. of branches		0.08	-0.45	0.00	-0.35	X						
Number of sprouts		-0.40	-0.34	0.00	0.17	0.67	0.74	X				
Length of sprouts		0.19	0.13	0.00	-0.21	0.93	0.75	0.38	0.54	X		
Color range												
From		0.9	0.7	0.5	0.3	0.0	-	0.0	-0.3	-0.5	-0.7	-0.9
Up to		1.0	0.9	0.7	0.5	0.3	-	-0.3	-0.5	-0.7	-0.9	-1.0

*Horizontal branches are represented by non-italic text; vertical branches are presented by italic text; The range of values (positive/negative) indicate a Pearson's correlation of: 0.0 to 0.3 very weak or negligible; 0.3 to 0.5 weak; 0.5 to 0.7 moderate; 0.7 to 0.9 strong; 0.9 to 1.0 very strong.

Rooting performance of canopy and epicormic cuttings

Significant differences between treatments were detected for survival, callus formation and sprouting variables according to ANOVA (Table 2). Canopy-derived cuttings presented higher survival (72.9%) compared to epicormic cuttings (45.8%). Similarly, callus formation reached 65.6% in canopy material, whereas epicormic cuttings presented a value more than 50% lower, of 11.5%.

The production of new sprouts followed the same pattern, with canopy cuttings presenting approximately 38.5% sprouting compared to 9.4% in epicormic material. Rooting percentages did not differ significantly between origins, with averages of 36.5% for canopy cuttings and 34.4% for epicormic cuttings. Retention of original leaves also showed no significant differences between treatments, remaining above 40% for both material sources.

DISCUSSION

Influence of disposition sense on epicormic sprouting dynamics

This study expands the findings previously reported by Nascimento *et al.* (2023), where the inclusion of additional variables such as branch diameter and disposition sense were suggested to better understand epicormic sprouting responses in *Ilex paraguariensis*. The present results demonstrate that disposition sense was the primary factor influencing sprouting dynamics, affecting both the temporal pattern of bud activation and the longevity of detached branches.

Horizontally disposed branches exhibited slower sprouting development, with maximum sprouting values around 50%, but maintained viable sprouts for longer periods, suggesting a more gradual use of internal reserves.

Table 2: Rooting variables of *I. paraguariensis* cuttings rescued from tree canopy sprouts and epicormic sprouts produced from detached branches.

Origin	S	C	R	SP	OL
	----- % -----				
CP	72.9 a* ± 2.5†	65.6 a ± 3.8	36.5 ^{ns} ± 5.6	38.5 a ± 4.8	58.3 ^{ns} ± 6.2
EP	45.8 b ± 2.6	11.5 b ± 2.6	34.4 ^{ns} ± 4.5	9.4 b ± 3.8	42.7 ^{ns} ± 2.9
Av.	59.4	38.5	35.4	24.0	50.5
p	< 0.0001	< 0.0001	0.8076	0.0003	0.0986
CV%	7.0	18.9	47.4	51.6	35.0

*Averages followed by different letter differ significantly according to the Student's t test ($p < 0.0050$); ns non-significative averages; † Standard error; S: Survival; C: Callus formation; R: Rooting; SP: Sprouting; OL: Original leaves; CP: Canopy; EP: Epicormic; Av.: Average; CV%: Coefficient of variation in percentage.

Similar patterns have been described for detached branches of *I. paraguariensis* (Nascimento *et al.*, 2018), *Drimys brasiliensis* (Silva *et al.*, 2023), and *Moquiniastrium polymorphum* (Schatz Sá *et al.*, 2024), where sprouting occurred progressively and produced relatively short sprouts during early evaluations. These findings reinforce the interpretation that horizontal positioning may prolong physiological activity while limiting rapid sprout emergence.

In contrast, vertically positioned branches showed faster initial sprouting responses but reduced longevity, as survival declined earlier compared to horizontal arrangements. Previous studies have reported higher sprouting percentages and larger numbers of epicormic sprouts in vertically disposed branches (Nascimento *et al.*, 2023; Silva *et al.*, 2023; Schatz Sá *et al.*, 2024), including in *I. paraguariensis* (Wendling *et al.*, 2013). However, the lower values observed in the present study indicate that disposition alone does not fully explain sprouting success and suggests the involvement of additional biological or environmental factors.

One possible explanation involves differences in internal hormonal transport and sap flow. Maintaining branches in their natural vertical orientation may facilitate auxin and cytokinin redistribution toward dormant buds, promoting faster bud activation (Hartmann *et al.*, 2018; Taiz *et al.*, 2023). Conversely, horizontal positioning may alter hydraulic continuity and require alternative transport pathways, potentially delaying bud activation while extending branch lifespan. Although hormonal dynamics were not directly measured, this physiological framework provides a plausible interpretation consistent with previous literature.

Role of genotype, ontogenetic condition and material selection

The variability observed among treatments indicates that genetic background and ontogenetic factors may have influenced epicormic sprouting responses. Studies with other species, such as *Cordia trichotoma* and *Paratecoma peroba*, have demonstrated strong genotype-dependent variation in epicormic sprouting productivity, even under

standardized experimental conditions (Silva *et al.*, 2021a; Silva *et al.*, 2021b). In addition, ontogenetic age is known to affect bud responsiveness and vegetative vigor, with higher vigor tissues generally presenting higher sprouting potential (Hartmann *et al.*, 2018; Duarte *et al.*, 2025).

In the present study, branches were collected within a standardized height range to reduce ontogenetic variation; however, genetic differences among selected trees may still have contributed to the variability in sprouting performance. Future studies incorporating genotype as an experimental factor could clarify this influence.

Another relevant methodological aspect is branch morphology. Unlike previous studies that included bifurcated or knot-bearing branches (Nascimento *et al.*, 2023), the present experiment prioritized morphological standardization by selecting straight, non-bifurcated materials. Although this approach reduced variability, it may also have limited the number of dormant buds available, potentially contributing to the lower sprouting values observed.

Effects of branch diameter on sprouting responses

Branch diameter showed secondary and context-dependent effects when compared to disposition sense. In general, thinner branches displayed earlier sprouting responses regardless of positioning, suggesting faster physiological activation. This pattern may be related to shorter distances between dormant buds and the bark surface, potentially facilitating bud emergence, as described for other vegetative rescue techniques such as girdling (Hartmann *et al.*, 2018).

However, diameter did not consistently affect branch survival, particularly in horizontally disposed treatments, indicating that stored reserves may not be strictly proportional to branch thickness. Instead, the interaction between physiological activity and energy consumption may better explain the observed patterns. Vertically disposed thin branches showed reduced longevity, possibly due to faster reserve depletion associated with rapid sprouting activity, a trend also suggested by Silva *et al.* (2023) and Schatz Sá *et al.* (2024).

Further investigations including additional diameter classes and anatomical analyses would help clarify the relative contribution of structural and physiological factors.

Temporal decline in sprouting productivity

Regardless of treatment, a decline in sprouting activity and productivity was observed over time. This pattern has been consistently reported in studies involving detached branches (Wendling *et al.*, 2013; Nascimento *et al.*, 2018; Lima *et al.*, 2020; Silva *et al.*, 2023; Schatz Sá *et al.*, 2024) and is likely associated with progressive depletion of carbohydrate reserves and the absence of continuous physiological support from the selected tree. Therefore, detached branches should be considered a temporary propagation strategy, requiring optimization of harvest timing to maximize sprout quality and availability.

Performance of epicormic sprouts as propagation material

Even though the use of epicormic sprouts in vegetative propagation across diverse woody taxa is a common subject of research, only a limited number of studies have evaluated epicormic sprouts produced from detached branches as a propagation source (Wendling *et al.*, 2013; Nascimento *et al.*, 2018; Lima *et al.*, 2020; Silva *et al.*, 2021a; Silva *et al.*, 2021b; Silva *et al.*, 2023; Schatz Sá *et al.*, 2024; Maqbool and Aftab, 2024). The present results demonstrate that epicormic sprouts from detached branches can achieve rooting percentages statistically comparable to canopy-derived material, reinforcing their potential as an alternative vegetative rescue strategy.

On the other hand, epicormic cuttings showed reduced survival and callus formation compared to canopy material. Similar variability among species has been reported in previous studies, highlighting the importance of species-specific physiological responses (Silva *et al.*, 2023; Schatz Sá *et al.*, 2024). Differences in rooting dynamics among propagules may reflect variations in physiological activity and root induction speed, as previously observed in *Eucalyptus* mini-cuttings subjected to distinct propagation conditions (Brondani *et al.*, 2012). For *I. paraguariensis*, Wendling *et al.* (2013) reported higher survival and rooting rates than those observed here, also suggesting that environmental conditions and sprout developmental stage may strongly influence propagation success.

Influence of lignification and environmental conditions

Environmental conditions during epicormic sprouting may strongly influence their morphology and subsequent rooting performance, as demonstrated for detached branches and epicormic sprouts of woody species subjected to vegetative rescue techniques (Silva *et al.*, 2021a). In this matter, the mini-tunnel system used in this experiment may have contributed to the rapid development of epicormic sprouts, potentially producing less lignified tissues more susceptible to stress during rooting. According

to Xavier *et al.* (2013) and Hartmann *et al.* (2018), propagule lignification plays a key role in reducing water loss and improving survival during vegetative propagation.

Comparisons with studies involving epicormic sprouts induced by girdling or coppicing further support this interpretation. Longer developmental periods prior to harvesting have been associated with improved survival and rooting performance (Stuepp *et al.*, 2017; Nascimento *et al.*, 2018), possibly due to increased tissue maturation.

Nevertheless, rooting responses may vary according to species and physiological status. While some studies suggest that less lignified propagules possess higher endogenous hormone levels and rooting potential (Maqbool and Aftab, 2024), others report superior results with more lignified materials (Silva *et al.*, 2023), indicating that an optimal balance between juvenility and structural maturity may be required. Furthermore, environmental conditions during propagation can strongly modulate rooting responses and propagule survival (Nascimento *et al.*, 2022).

Ontogenetic considerations and implications for propagation

Ontogenetic age remains a key factor influencing vegetative propagation success (Hartmann *et al.*, 2018). In woody species, the transition from juvenile to adult phases involves progressive reductions in morphogenic competence, which directly affect adventitious rooting capacity (Wendling *et al.*, 2014a). Although epicormic sprouts derived from detached branches may exhibit increased physiological activity, they should not be considered fully rejuvenated tissues. Instead, these propagules reflect a condition of reinvigoration, in which there is a partial recovery of vigor without a complete reversion to the juvenile ontogenetic phase (Wendling *et al.*, 2014b; Stuepp *et al.*, 2018). This distinction is essential, as vegetative rescue techniques have been described as promoting rejuvenation and/or reinvigoration effects, depending on the propagation strategy and biological context (Stuepp *et al.*, 2018).

This distinction is particularly relevant for interpreting the present results, since the similar rooting percentages observed between epicormic and canopy-derived cuttings suggest that, despite differences in physiological status, epicormic sprouts maintained sufficient morphogenic competence for root induction. Reinvigorated propagules may temporarily recover morphogenic competence even without complete ontogenetic rejuvenation (Wendling *et al.*, 2014b). However, the reduced survival and callus formation observed in epicormic material indicate that reinvigoration alone may not be sufficient to fully overcome limitations imposed by tissue maturation.

Overall, detached branches represent a promising *ex situ* strategy for producing propagation material without causing severe damage to selected trees, supporting applications in conservation and clonal forestry programs (Wendling and Brondani, 2015). For *I. paraguariensis*, previous studies have demonstrated that propagation success depends not only on the origin of the propagules

but also on their physiological and ontogenetic condition, as well as environmental management during rooting (Wendling *et al.*, 2005; Stuepp *et al.*, 2017). Furthermore, the use of vegetative propagation techniques has been associated with increased productivity and uniformity in commercial plantations, reinforcing the relevance of developing efficient propagation strategies based on physiologically competent propagules (Wendling and Brondani, 2015). Therefore, optimizing factors related to reinvigoration and propagule quality remains essential to improve the efficiency of vegetative rescue techniques.

CONCLUSIONS

Detached branches proved to be a viable *ex situ* source for epicormic sprout production in *Ilex paraguariensis*, with sprouting dynamics being primarily influenced by branch disposition sense rather than diameter. Vertically positioned branches promoted faster initial sprouting but reduced longevity, whereas horizontally positioned branches showed slower sprouting development combined with extended survival, indicating that propagation strategies may be adjusted according to the desired balance between productivity and consistency of material supply. Epicormic sprouts derived from detached branches exhibited rooting performance comparable to canopy-derived material under the evaluated conditions, supporting their potential use as propagation materials. However, lower survival rates indicate the need for further optimization of environmental conditions and propagule physiological status. Overall, detached branches represent a promising alternative for vegetative rescue and propagation of *I. paraguariensis*, although additional studies are still required to refine the technique and improve propagation efficiency.

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AUTHORSHIP CONTRIBUTION:

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Database: BN; ACSS; TS; CM
Processing: BN; ACSS; TS; CM; MCN
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Data availability:

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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