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A broad analysis of vegetative rescue and propagation of *Moquiniastrum polymorphum* (Less.) G. Sancho

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Abstract

Background: The primary method for propagating forest species is through seeds, which is cost-effective and ensures genetic adaptability to environmental changes. However, germination issues and genetic variability can hinder standardisation of productivity. In the case of *Moquiniastrum polymorphum*, a species known for its remarkable wood quality and pharmacological potential, seedling production and genetic improvement efforts have been limited. This study focused on the rescue and vegetative propagation of *M. polymorphum*, which are crucial steps for species selection and genetic enhancement.

Methods: Protocols were tested to rescue and propagate propagules from different individuals collected in Lages, Santa Catarina (2020/2022). The vegetative rescue tests were: (I) epicormic sprouting induction through girdling techniques and detached branches; and (II) influence of individuals, disposition sense and time on the epicormic sprouting of detached branches. Vegetative propagation analyses included: (I) cutting according to individuals; and (II) relation between rooting environments and individuals on cutting.

Results: The results indicated that the girdling techniques were not efficient for vegetative rescue, as only 8% of individuals produced epicormic sprouts. In contrast, detached branches showed a much higher success rate of 80% for epicormic sprouting, revealing significant differences in sprout development among individuals over time. Vertically oriented branches produced nearly three times more sprouts compared to horizontally oriented branches. Regarding vegetative propagation, certain individuals exhibited remarkable rooting rates of over 75%. However, no conclusive results were obtained when using epicormic materials or when considering different rooting environments.

Conclusions: Given the significance of rescue and vegetative propagation in plant genetic improvement and the limited research addressing these aspects in *M. polymorphum*, this study holds substantial importance for future investigations. It is recommended to expand rescue and vegetative propagation studies to encompass additional populations, different individuals, and rooting environments, further advancing our understanding and efforts to enhance this species.

Keywords: cambará, detached branches, girdling, rooting environment

Introduction

Moquiniastrum polymorphum (Less.) G. Sancho (Asteraceae), previously known as *Gochnatia polymorpha*, is a native tree species found in various forest typologies in Brazil, particularly in the Cerrado biome, Ombrophilous Forest, and Mixed Ombrophilous Forest. From an ecological perspective, it is important in both primary and secondary succession processes and thrives in nutrient-poor areas, making it a recommended choice for reforesting degraded regions (Lorenzi 2014). It is valued for its wood, which is moderately heavy, hard, and compact, rendering it well-suited for challenging environmental conditions (Faria et al. 2019). Furthermore, recent research suggests notable pharmacological potential, as extracts derived from its leaves have demonstrated phytotoxic effects on other plants (Pinto & Kolb 2015) and are being explored as alternative treatments for inflammatory ailments (De Moraes et al. 2019) and oxidative diseases (Bastos et al. 2022).

Despite its ecological and economic significance, *M. polymorphum* has a low germination rate. Even though seed dormancy does not occur, germination is still reported to be relatively low, ranging between 30% and 50% (Lorenzi 2014). Within the Asteraceae family, germination issues are common, and this may be attributed to factors such as seed emptiness (Chaves & Ramalho 1996). Moreover, there have been few studies on seed propagation of *M. polymorphum* (Faria et al. 2019), regardless of the importance of this method for natural selection and adaptation to adverse environmental conditions over time.

In addition to low germination rates, producing plants from seeds does not guarantee the desired silvicultural characteristics because it involves cross-fertilisation and results in completely new individuals with unique genetic makeups. In order to achieve standardisation in growth and production, the implementation of vegetative propagation techniques is necessary. Vegetative propagation takes advantage of the totipotency of plant cells, theoretically allowing the production of an entire new plant from a single vegetal cell (Xavier et al. 2013). These techniques enable direct selection of individuals with desired traits, establishing clonal gardens to produce a large quantity of individuals (Oliveira et al. 2016). However, there is currently very little research on the use of vegetative propagation for *M. polymorphum*, despite its potential for crucial vegetative improvement of the species.

Several factors directly influence the success of vegetative propagation, particularly when using a cutting technique. This method is efficient and straightforward, as it enables the selection of mature individuals and the production of significant quantities of new seedlings within relatively short timeframes (Xavier et al. 2013). Some of these influential factors include: (i) selecting the correct individual; (ii) overcoming the maturation of the mother plant; and (iii) determining the optimal rooting environment.

Firstly, rooting potential varies with genotype (Pimentel et al. 2019). Several studies have shown contrasting percentages of rooting among propagules from different genotypes within the same population (Nascimento et al. 2020), as well as among established clones (Vieira et al. 2021). On the other hand, determining such characteristics can be challenging, as they are primarily influenced by genetic and physiological factors rather than easily identifiable phenotypic traits. This complexity may hinder the process of direct selection.

Desired phenotypic characteristics in forest species often become more pronounced after certain levels of maturity of the mother plant. This poses a challenge for material collection and propagation (Wendling et al. 2014; Stuepp et al. 2018; Nascimento et al. 2018). Nonetheless, this maturation process can be reversed by using juvenile material, also referred to as ontogenetic young, which is commonly found in the dormant buds located in the lower parts of the plant's stem. Specific techniques can be applied to stimulate the development of these buds into new sprouts, enabling their collection and subsequent propagation (Stuepp et al. 2015; 2017; Nascimento et al. 2018).

One common technique to induce the production of epicormic sprouts, considered as reinvigorated material, is stem girdling, which involves interrupting the sap flow and creating an imbalance between auxin and cytokinin. This disruption promotes the development of buds below the girdled area (Santin et al. 2008). Another technique involves the use of detached branches, which are stored under optimal greenhouse conditions to stimulate epicormic sprouting. This approach facilitates subsequent steps in vegetative propagation (Wendling et al. 2013). Although not generally recommended, even ontogenetic old material, such as sprouts from canopies of trees, can still exhibit significant rooting when subjected to ideal conditions (Stuepp et al. 2017; Nascimento et al. 2020;2022).

Another crucial factor for successful vegetative propagation is the rooting environment. An efficient rooting environment should maintain a stable temperature range with minimal fluctuations and provide constant high humidity throughout the rooting process (Xavier et al. 2013). However, there is a limited number of current studies that specifically assess this variable for native tree species. In contrast to exotic forestry species like the *Eucalyptus* genus, the research on rooting environments for native species in Brazil is relatively scarce. Moreover, it's important to note that environments with temperature and humidity control, tailored to specific species, may not be suitable for others, as each species has its own unique requirements and characteristics (Nascimento et al. 2022).

Given the information presented above, several important questions arise. These questions include: Are there variations in vegetative rescue and propagation based on different individuals? Is the use of reinvigorated materials necessary? Can certain rooting environments be more suitable than others? Therefore, the objective of this study was to investigate the vegetative rescue of the species using girdling and detached branch techniques, as well as its propagation through cuttings, taking into consideration individuals and rooting environments.

Methods

Population determination and individual selection

The studied individuals were located in an experimental site of the University of the State of Santa Catarina (UDESC) in the municipality of Lages, Santa Catarina state (27°45'39" S and 50°04'36" W). The forest type is characterised as Mixed Ombrophilous Forest, also known as Araucaria Forest, with characteristics of a secondary forest in different stages of succession. According to the Köppen's classification, the climate of the region is classified as *Cfb*, with a temperate climate and mild summers. Rainfall is evenly distributed throughout the year, without a dry season. The region has an average annual temperature of 17.0 °C and an average annual precipitation of 1,500 mm.

A forest inventory was previously conducted in the experimental area, mapping a total of 302 individuals of *M. polymorphum*. Among them, some criteria were used to select those for the experiments. Firstly, only individuals with a diameter at breast height (DBH) greater than 5 cm were selected, based on the higher availability of plant material, and ensuring their resilience in the application of abrasive treatments. Diameters were obtained using a measuring tape around the circumference at breast height (CBH, 1.3 m), which was then divided by π to obtain the DBH value. Secondly, after determining desired diameters, individuals with the greatest possible distance between them were chosen to ensure higher genetic diversity within the population of the species. Finally, only individuals free from pests and diseases, and phenotypically similar were included in the final selection. Hence, 26 individuals of *M. polymorphum* were identified for the vegetative rescue and propagation experiments (Figure 1 and Table 1). It is emphasised that the selection of individuals was carried out randomly, in accordance with the previously mentioned guidelines.

Two main items were considered in the experimental layout. The first part of the study involved the application of rescue techniques using the production of epicormic sprouts, and the experiments in this section consisted of: (I) epicormic sprouting induction through girdling techniques and detached branches; and (II) influence of individuals, disposition sense and time on the epicormic sprouting of detached branches. The second part focused on vegetative propagation using cuttings. This portion of the study involved: (I) exploratory analyses considering individuals and epicormic material; and (II) cutting according to rooting environments and individuals.

1. Epicormic sprouting induction through girdling techniques and detached branches

Nineteen *M. polymorphum* individuals were submitted to three different vegetative rescue techniques in September 2020. These techniques were: complete girdling (100% of the bark circumference), semi-girdling (50% of the bark circumference), and collection of detached branches.

The complete girdling technique was applied to the following individuals: 2, 4, 47, 68, 80, and 116, totaling six individuals. This technique involves the complete removal of the bark in a 5 cm wide strip around the circumference of the stem (100% of a ring), starting

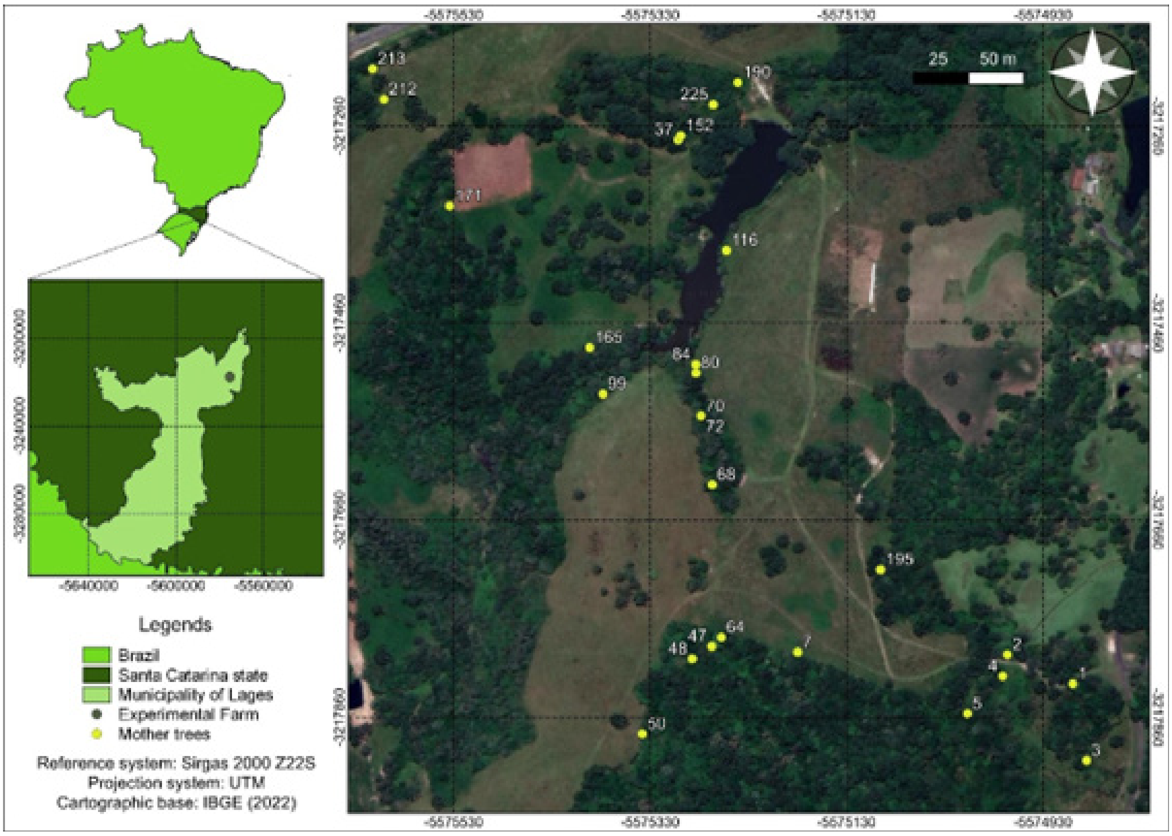


FIGURE 1: Location map of the experimental farm of the University of Santa Catarina state in Lages, Santa Catarina state, and the location of 26 out of 302 *M. polymorphum* selected trees.

TABLE 1: Dendrometric variables of each *M. polymorphum* individual tested in different vegetative rescue and propagation experiments.

***M. polymorphum* Individual**		**Property**		**Testing regime(s)**[3]
Number	**Identification code**	**CBH[1] (cm)**	**DBH[2] (cm)**	
N. 1	1	153.7	48.9	*Rb*
N. 2	2	115.5	36.8	*Rg, Rb*
N. 3	3	159.8	50.9	*Gn, Re*
N. 4	4	132.0	42.0	*Rg, Rb, Gn*
N. 5	5	58.5	18.6	*Rs*
N. 6	7	21.5	6.8	*Gn, Re*
N. 7	37	160.0	50.9	*Rs, Rb*
N. 8	47	98.0	31.2	*Rg*
N. 9	48	54.0	17.2	*Rs*
N. 10	50	159.8	50.9	*Gn, Re*
N. 11	64	85.0	27.1	*Gn, Re*
N. 12	68	79.0	25.1	*Rg, Rb, Gn, Re*
N. 13	70	58.0	18.5	*Gn, Re*
N. 14	72	49.8	15.9	*Rs, Rb*
N. 15	80	166.4	53.0	*Rg, Gn, Re*
N. 16	84	103.0	32.8	*Rs, Rb, Gn, Re*
N. 17	99	16.6	5.3	*Gn*
N. 18	116	32.1	10.2	*Rg*
N. 19	152	32.3	10.3	*Gn, Re*
N. 20	165	18.9	6.0	*Gn*
N. 21	171	52.5	16.7	*Gn, Re*
N. 22	190	64.6	20.6	*Gn, Re*
N. 23	195	142.0	45.2	*Rs*
N. 24	212	38.5	12.3	*Gn*
N. 25	213	36.0	11.5	*Gn, Re*
N. 26	225	40.9	13.0	*Gn*

[1] CBH = Circumference at breast height in centimeters
[2] DBH = Diameter at breast height in centimeters
[3] *Rb* = Vegetative rescue using detached branches; *Rg* = Vegetative rescue using complete girdling; *Rs* = Vegetative rescue using semi-girdling; *Gn* = Vegetative propagation according to individuals; and *Re* = Vegetative propagation according to the rooting environment.

from 20 cm above the soil level. Careful attention was given to ensure that only the superficial part of the bark was removed, without damaging the underlying wood, with a well-sharpened machete.

The semi-girdling technique was applied to the following individuals: 1, 5, 37, 68, 72, and 84, totalling six individuals. This technique followed the same principles as the complete girdling technique, with the only difference being that only half of the length of the bark ring was removed (50% of the ring). The procedure ensured that the bark was carefully removed along a specific section of the stem circumference, while leaving the other half intact.

Detached branches were collected from a total of seven individuals: 1, 2, 4, 37, 68, 72, and 84. For each individual, three branches were carefully selected and pruned using a chainsaw, at an estimated height varying from 1.5 m to 2.5 m. The collected branches, which were between 30 cm and 50 cm long, were then prepared by removing any remaining sprouts and leaves. To prevent excessive moisture loss, the branches were placed in a Styrofoam box filled with cooled water. Then they were transported to the forest nursery of the University of Santa Catarina State, located in Lages, Santa Catarina state. Upon arrival at the nursery, the branches were vertically staked into containers filled with fine vermiculite to provide support. The upper cut section of the branches was sealed with plastic film, aiming to prolong their lifespan. To create an optimal environment for sprouting, all material was kept in a mini tunnel system inserted in a shaded greenhouse (50%). The mini tunnel system employed micro sprinkler irrigation, which consisted of ten irrigation cycles lasting ten minutes each.

Following the girdling treatments, sprouting success was checked at 384 days. For the detached branches, a single evaluation was conducted at 70 days after the treatment application. The variables examined included tree or branch survival percentage, sprouting percentage, number of sprouts, and length of sprouts in centimeters.

To determine tree or branch survival, living trees were identified as those that showed no signs of decay or deterioration, such as leaf loss or drying symptoms, despite the initial injury caused by the girdling technique. Similarly, living branches were recognised by their unchanged coloration, absence of fungal growth, and lack of drying conditions. Effective sprouting for trees was determined by the presence of new sprouts measuring at least 0.5 cm in length below the girdled area. For branches, any new sprouts of at least 0.5 cm, regardless of their position, were considered as evidence of effective sprouting. Only sprouts directly originating from the tree's or branch's stem were counted when assessing the number of sprouts. Secondary sprouts that emerged from primary sprouts were not included in the count. The length of sprouts was measured using either a millimeter ruler or a millimeter tape, ensuring accurate measurements of the sprout's elongation.

The experiment was carried out using a completely randomised design, which included three different vegetative rescue treatments: complete girdling, semi-girdling, and collection of detached branches. Each tree subjected to the girdling techniques was considered as a repetition, resulting in six repetitions for each treatment. In the case of the detached branches, three branches were collected from each tree, and the average of the three branches was considered as a single repetition. Therefore, there were a total of seven repetitions for the detached branches treatment.

2. Influence of individuals, disposition and time on the epicormic sprouting of detached branches

From the previously mentioned *M. polymorphum* individuals, an additional two sets of three branches were collected from each individual during the same collection period. The branches were subjected to the same transportation and preparation procedures as described earlier, and they were kept under the same climatic conditions within the mini tunnel system. Furthermore, the same variables under the same conditions were also assessed.

In this experiment, three different conditions were evaluated in relation to the sprouting of the branches. The first condition was the effect of the different individuals on the sprouting response. The second condition examined the influence of the disposition of the branches on sprouting, considering branches maintained in the vertical and horizontal positions. For the branches maintained horizontally, those were laid over containers of tubes, avoiding direct contact with the ground. For this condition, both sides of these branches were sealed with plastic film. Lastly, the evaluation periods were another factor that was assessed. The branches were evaluated every ten days for a total of nine assessments.

This experiment followed a completely randomised design and involved two patterns of evaluation. In the first pattern, a factorial scheme of 7 x 2 was employed. Factor A comprised the seven individuals that were tested, while factor B represented the two different branch dispositions: vertical and horizontal. For this condition, a single evaluation was considered at 70 days after the experiment implementation. In the second pattern, a factorial scheme of 2 x 9 was implemented. Factor A represented the two branch dispositions (vertical and horizontal), and factor B comprised the evaluation periods, with measurements taken at intervals of ten days. The population values were considered in this condition.

3. Exploratory analyses considering individuals and epicormic material

In the second part of the study three experiments were conducted concurrently in November 2020 to investigate the rooting potential of *M. polymorphum* cuttings. The aim was to understand the factors of individuals and ontogenetic age influencing rooting success and to optimise the vegetative propagation of the species, taking into consideration two simple analyses.

Experiment I aimed to evaluate the rooting ability of cuttings derived from different individuals. Canopy sprouts were selected as the source material for the cuttings. Material was collected from a total of 17 individuals, at an estimated heigh varying from 1.5 m to 2.5 m, represented by the following individuals: 3, 4, 7, 50, 64, 68, 70, 80, 84, 99, 152, 165, 171, 190, 212, 213 and 225. These individuals were considered as a population sample for the subsequent experiment.

Experiment II involved a comparison between cuttings derived from canopy sprouts and epicormic sprouts obtained from detached branches. The epicormic sprouts were provided by the previously experiments of detached branch tests, approximately 90 days after the previous test installation. The aim was to assess whether there were any differences in the rooting potential between these two types of cuttings, considering their ontogenetic age, with canopy sprouts representing older growth and epicormic sprouts representing younger growth. For canopy material, the population results obtained from Experiment I were considered.

The sprouts, whether obtained from the canopies of the trees or the detached branches, were collected using pruning shears. Sprouts collected from the canopy were carefully stored in a Styrofoam box filled with cooled water to prevent excessive moisture loss during transportation. These boxed sprouts were then transported to the forest nursery of the University of Santa Catarina state. Epicormic sprouts, on the other hand, were obtained from the branches that were already present and maintained at the nursery. These sprouts were collected on-site and processed immediately.

To prepare the sprouts for rooting, they were carefully sectioned into cuttings using a bevel cut of approximately 8 cm (with a tolerance of ± 2 cm). During the sectioning process, one or two leaves were retained, with a reduction of approximately 50% of the total leaf area. This was done to prevent excessive transpiration. To ensure the quality and standardisation of the cuttings, the apical part and/or the most lignified sections of the sprouts were discarded. This step aimed to provide uniformity in the sectioned material used for the experiment. Prior to implantation in the substrate cuttings were kept in cooled water to maintain their hydration and prevent desiccation.

The prepared cuttings were inserted into tubes with a volume of 180 cm^3. These tubes were filled with a commercial substrate consisting of a mixture of peat, vermiculite, organic waste classified as "A," and limestone. The substrate had a density of 130 kg m^{-3}, a water retention capacity of 300% m/m, and a pH of 5.5. To provide essential nutrients for the development of the rooted cuttings, 6 g L^{-1} of a controlled-release fertiliser with a composition of 15-9-12 (NPK) was added to the substrate. This addition aimed to ensure the continuous supply of nutrients during the rooting and subsequent growth stages of the cuttings. All trays containing the cuttings were placed in the mini tunnel system mentioned earlier.

The following variables were evaluated after 90 days for both experiments in percentage: survival, callus formation, rooting, presence of new sprouts, and retention of original leaves. The percentage values for all variables were calculated based on the total number of cuttings used in the experiments. Live cuttings were defined as those demonstrating signs of vitality, including live wood, mature leaves, or young sprouts, irrespective of their successful rooting outcome. Callus formation was ascertained by the presence of an undifferentiated mass of cells at the base of the cuttings, regardless of their rooting status. Rooted cuttings were identified when they exhibited the development of root primordia measuring at least 5.0 mm in length. Cuttings with new sprouts were characterised by the emergence of fresh leaves subsequent to the cutting process. The assessment of original leaves involved determining their continued presence on the cuttings at the time of evaluation.

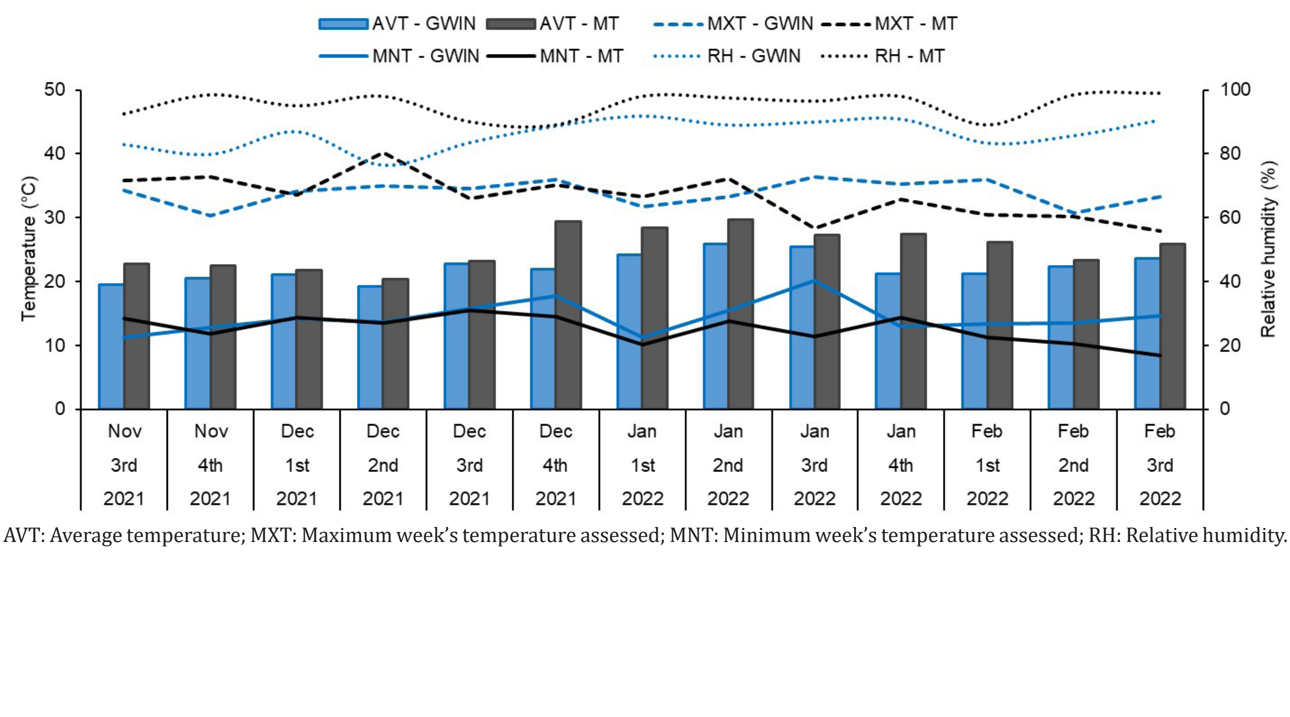
Both experiments conducted in this section followed a completely randomised design, each with its specific response variables. In experiment I the focus was solely on the cutting process of the 17 individuals. Each individual was represented by 18 repetitions, and within each repetition, nine cuttings were assessed, resulting in a total of 162 cuttings per individuals. Experiment II involved the comparison between canopy and epicormic material obtained from detached branches. Due to the limited availability of suitable sprouts for cutting in the detached branches, the number of repetitions was reduced to six. Within each repetition, seven cuttings were examined, resulting in a total of 42 cuttings.

4. Cutting according to rooting environments and individuals

In the third part of the study the rooting of *M. polymorphum* cuttings was tested using seven individuals: 3, 7, 50, 64, 80, and 190; and two distinct rooting environments. The two rooting environments assessed were as follows (Figure 2):

(I) Mini Tunnel System (MT): The MT was a simplified structure located within a shade house at the Forest Nursery of UDESC in Lages, Santa Catarina state (this structure was previously mentioned, used for storing the detached branches in a previous methodology). The MT featured automatic irrigation using micro sprinklers, with four daily irrigation sessions scheduled at 9:00 AM, 12:00 PM, 3:00 PM, and 6:00 PM. Each irrigation session lasted for ten minutes. The characteristics and setup of the MT were previously described by Pereira et al. (2019).

FIGURE 2: Temperature (°C) and relative humidity (%) of the mini tunnel system (MT) and the greenhouse with intermittent nebulisation (GWIN) during the weeks of experiment of *M. polymorphum* individuals cuttings.



AVT: Average temperature; MXT: Maximum week's temperature assessed; MNT: Minimum week's temperature assessed; RH: Relative humidity.

(II) Greenhouse with Intermittent Nebulization (GWIN): The GWIN represented a more advanced technological structure situated in a forestry company located in the municipality of Otacílio Costa, Santa Catarina state. The GWIN was equipped with automatic temperature control, maintaining temperatures between 20 °C and 30 °C using a thermostat. The humidity levels in the GWIN were consistently maintained above 70.0%. The details and specifications of the GWIN system were previously documented by Nascimento et al. (2020; 2022).

All the plant material utilised in these analyses was collected from the canopies of the trees in November 2021. The transportation, preparation of the material, establishment of the experimental area, and evaluation of the variables followed the same protocols and procedures as the previous cutting experiments. After material preparation, one set of repetitions of each individual was transported to GWIN. During transportation, the material was frequently spayed with water to avoid its excessive transpiration.

This analysis followed a completely randomised design with a factorial scheme of 7 x 2. The factor A represented the seven individuals tested, and the factor B represented the two rooting environments (MT and GWIN). Each individual was represented by six repetitions, and each repetition consisted of nine cuttings, resulting in a total of 54 cuttings for each rooting environment.

Statistical analyses

All data were subjected to preliminary analyses to ensure they met the assumptions required for statistical tests and, if required, a Box-Cox transformation was used. A Shapiro-Wilk test ($P > 0.05$) was conducted to assess normality of the data, while a Bartlett test ($P > 0.05$) was used to examine homogeneity of variance. Once these assumptions were confirmed, analysis of variance (ANOVA, $P < 0.05$) was performed. For experiments with a single factor, One-way ANOVAs were employed, while two-way ANOVAs were used for experiments with multiple factors. With statistical significance being met, post-hoc comparisons were conducted using the Scott-Knott test ($P < 0.05$). Additionally, for variables that involved multiple evaluations of the detached branches, a regression analysis of the dependent variables was performed, taking into consideration the adjusted R^2 value. The statistical software SISVAR, version 5.6 (Ferreira 2011), was utilised for all statistical analyses.

Summary of experiments

Figure 3 provides a visual representation that highlights the scope and design of each experiment, including the aspects evaluated and the evaluation time.

FIGURE 3: Vegetative rescue and propagation experiments of *Moquiniastrum polymorphum* assessing: (1) Epicormic sprouting induction through girdling techniques and detached branches; (2) Influence of individuals, disposition sense and time on the epicormic sprouting of detached branches; (3) Exploratory analyses considering individuals and epicormic material; and (4) Cutting according to adventitious rooting environments and individuals.

Session		Experiments	Evaluation time	Design
1	I	Complete girdling Semi-girdling Detached branches	→ For 384 days (3x) → At 70 days	→ Single factor Vegetative rescue techniques Population evaluated
2	I	Different individuals X Branches disposition (vertical and horizontal)	→ At 70 days	→ Factorial 7X2 1st factor = individuals 2nd factor = disposition
	II	Branches disposition (vertical and horizontal) X Evaluation periods	→ For 90 days (10x)	→ Factorial 2X9 1st factor = disposition 2nd factor = number of evaluations
3	I	17 individuals	→ At 90 days	→ Single factor 17 individuals (assessed separately)
	II	Materials source (canopy and branch sprouts)		→ Population evaluated
4	I	Different individuals X Rooting environment	→ At 90 days	→ Factorial 7 X 2 1st factor = individuals 2º factor = rooting environments

Results

1. Epicormic sprouting induction through girdling techniques and detached branches

The statistical analysis was not conducted in this experiment due to unfavorable results obtained from the girdling techniques. As a result, a meaningful comparison between treatments could not be performed (Table 2).

The selected trees underwent two assessments prior to the final evaluation, conducted at 32 and 209 days, respectively. However, no signs of sprouting were observed during these assessments. It was only during the final evaluation at 384 days that a single girdled mother tree with one epicormic sprout measuring approximately 47 cm was identified. In contrast, detached branches demonstrated a considerable potential for rescuing epicormic material, with over 80% of the branches sprouting. On average, each branch produced more than 6 sprouts measuring approximately 3 cm.

2. Influence of individuals, disposition and time on the epicormic sprouting of detached branches

None of the variables assessed showed significant differences for both factors ($P > 0.05$). However, individuals ($P = 0.0119$) and disposition ($P < 0.0001$) were significant for sprouting, while only individuals ($P = 0.0062$) was significant for the number of sprouts, and disposition ($P = 0.0001$) was significant for the length of sprouts (Table 3).

Sprouting was nearly six times more prevalent in vertically stored branches, with over 80% of branches exhibiting epicormic sprouts, compared to horizontally stored branches. Among the individuals, almost 80% of horizontally stored branches did not sprout, with only individuals 4 and 84 showing signs of sprouting. Furthermore, these two individuals displayed statistically superior sprouting compared to the others, with over 65% sprouting.

Regarding the number of sprouts, only individual 84 demonstrated superiority, with more than twice as many sprouts as individual 2. Although not statistically significant in the factorial scheme, it is worth noting that vertically stored branches had a higher number of sprouts compared to horizontally stored branches, with an average difference of nearly eight sprouts. Therefore, vertically stored branches facilitated almost nine times more effective sprout development compared to horizontally stored branches. These conditions are further illustrated when examining the sprouting and sprout development over time within the population (Figure 4).

Sprouting was observed at the 20-day evaluation in half of the materials from vertical branches, with an average of nearly 2 sprouts, but with a length of less than 1 cm. On the other hand, a single horizontally stored branch showed negligible sprouting at the third evaluation for both number and length.

Maximum sprouting was detected at 80 days for vertical branches, with 90% of the materials exhibiting sprouts. In contrast, maximum sprouting achieved for horizontal branches was 14% at 60 days, which remained constant until the final evaluation. The maximum average number of sprouts observed was 6.1 at the fifth evaluation for vertical branches, nearly three times higher than the maximum obtained for horizontal branches, which was 2.5 for the same period. Additionally, the maximum average length for vertical branches was 4.3 cm at 80 days, whereas the highest average for horizontal branches was almost eight times lower, at 0.6 cm on average during the same period.

3. Exploratory analyses of individuals and epicormic material

Considering the cutting of *M. polymorphum* individuals, all assessed variables showed statistically significant differences ($p<0.0001$). Notably, significant variations were observed among the 17 individuals that were tested (Table 4).

Individual 152 demonstrated exceptional performance, displaying superior values compared to other individuals. It achieved survival, and rooting rates close to 80%, indicating a high success rate in vegetative propagation. Almost 95% of the surviving cuttings of

TABLE 2: Survival, sprouting, number of sprouts and length of sprouts of *Moquiniastrum polymorphum* selected trees and detached branches according to three vegetative rescue techniques, in the municipality of Lages, Santa Catarina state, Brazil.

Rescue technique	Days of evaluation	Survival (%)	Sprouting (%)	Number of sprouts	Length of sprouts (cm)
	32	100	0.0	0.0	0.0
Girdling	209	100	0.0	0.0	0.0
	384	100	16.7	7.8	4.1
	32	100	0.0	0.0	0.0
Semi-girdling	209	100	0.0	0.0	0.0
	384	100	0.0	0.0	0.0
Branches	70	100	83.4 ± 6.2*	6.1 ± 0.3	3.4 ± 0.5

* Standard error.

TABLE 3: Sprouting, number of sprouts and length of sprouts of *Moquiniastrum polymorphum* detached branches from different individuals, in the municipality of Lages, Santa Catarina state, Brazil.

Parameter	**Individual**							
	1	**2**	**4**	**37**	**68**	**72**	**84**	**Average**
Disposition	Sprouting (%)							
Vertical	83.3†	100.0	100.0	33.3	66.7	100.0	100.0	83.3 a
Horizontal	0.0	0.0	33.3	0.0	0.0	0.0	66.7	14.3 b
Average*	41.7 b	50.0 b	66.7 a	16.7 b	33.4 b	50.0 b	83.4 a	48.8
Disposition	Number of sprouts							
Vertical	4.0	10.3	7.3	1.3	5.0	5.3	9.6	6.1
Horizontal	0.0	0.0	0.3	0.0	0.0	0.0	17.3	2.5
Average*	2.0 b	5.2 b	3.8 b	0.7 b	2.5 b	2.7 b	13.5 a	4.3
Disposition	Length of sprouts (cm)							
Vertical	7.1	1.4	4.4	0.7	1.8	4.5	4.3	3.5 a
Horizontal	0.0	0.0	0.5	0.0	0.0	0.0	2.1	0.4 b
Average*	3.6	0.7	2.5	0.4	0.9	2.3	3.2	1.9

*Averages followed by the same letter do not differ according to the Scott-Knott average test; † Standard error was not added due to the low number of repetitions.

this individual developed roots. Furthermore, by the end of the evaluation period, all living cuttings of individuals 152 had produced new sprouts, with nearly 80% retaining their original leaves.

Although individual 152 exhibited a high level of rooting success, a similar pattern of development was observed for most other individuals as well. Individuals 4, 7, 70, 84, 165, 190, 213, and 225, for instance, demonstrated a rooting success rate of at least 50% among the surviving cuttings. This suggests a substantial rooting capability within the *M. polymorphum* species, highlighting the need to optimise conditions for material survival. Additionally, the callus formation also followed this favorable pattern, indicating a high level of maturity in the materials used.

When examining the cutting performance based on the origin of the material, no statistically significant differences were observed ($p>0.05$) (Table 5). Overall, the survival rate reached approximately 22%, with half of the surviving cuttings successfully rooting. Only 5% of the living cuttings did not exhibit callus formation, while nearly 60% of the cuttings developed new sprouts and maintained their original leaves.

4. Performance of cuttings in relation to rooting environments and individuals

No significant difference was found between the individuals and rooting environments for any of the variables assessed (Table 6).

In general, individuals selected for this experiment showed lower survival and rooting performance compared to previous tests in this study. However, individuals 3, 7, and 190 exhibited the highest survival rates, all above 8%, at least two times higher than the others. This trend was also observed for callus formation and sprouting.

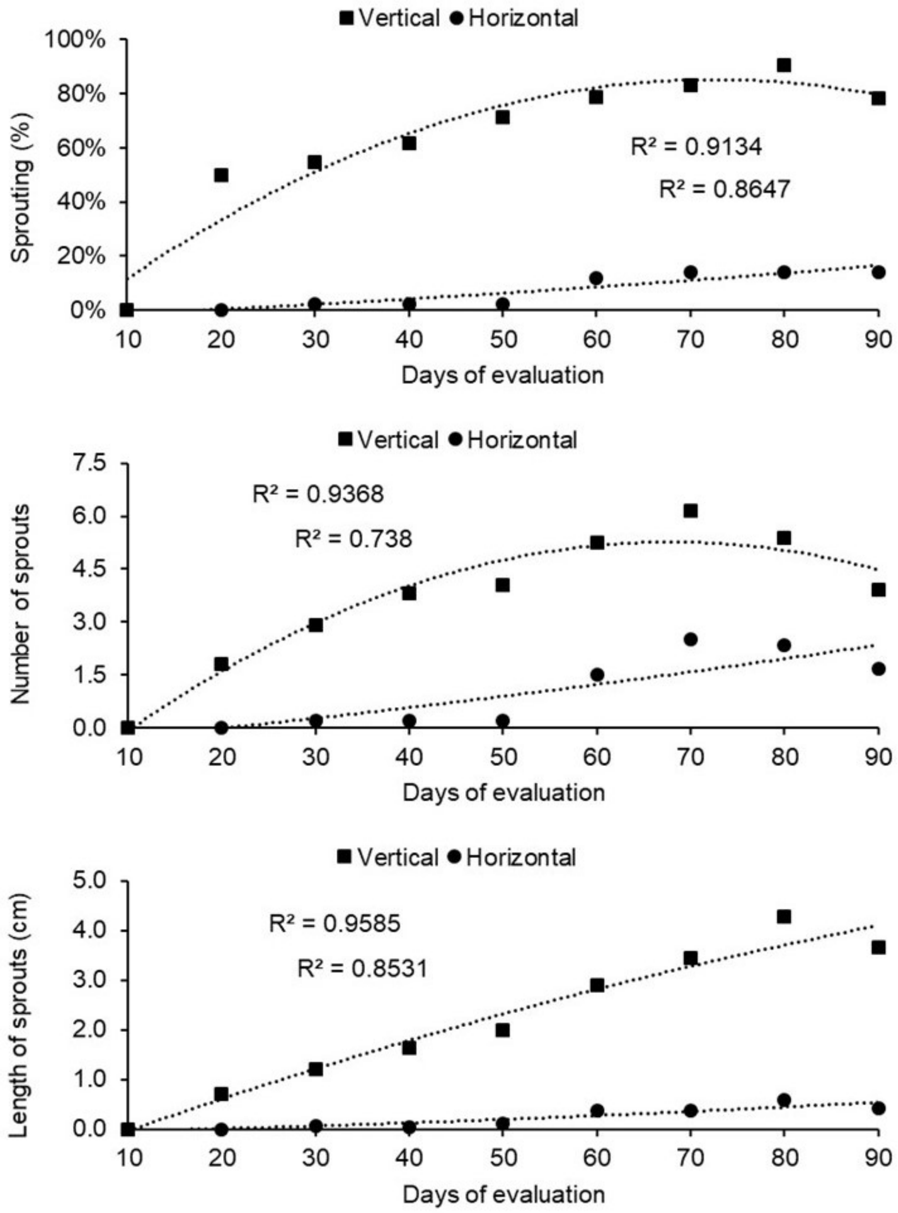
Discussion

1. Epicormic sprouting induction through girdling techniques and detached branches

Several factors could have contributed to the absence of sprouting through the girdling technique. These factors included inadequate injury to induce hormonal imbalance, the thickness of the bark hindering sprout development, or incorrect timing of the implementation. Additionally, the height of the injury and the number of dormant buds play a significant role. Stuepp et al. (2016) discovered that in *Ilex paraguariensis* St. Hill, sprouting was more successful when the bark was removed above 60 cm, resulting in nearly three times more sprouts compared to removal at 15 cm. The authors suggest that a greater surface area on the stem allows for more dormant buds, thereby facilitating more effective sprouting. This observation is supported by the sprouting observed in the detached branches in this study, which had a minimum length of 30 cm, resulting in a higher surface area and contact with dormant buds. Furthermore, dormant buds tend to accumulate as the plant grows taller (Hartmann et al. 2014), suggesting that there may have been an insufficient number of dormant buds below 20 cm in the girdled stems. This condition also explains the sprouting of the detached branches, which were collected between 1.5 m and 2.5 m.

Despite the absence of sprouting, the removal of bark did not cause harmful symptoms or lead to the mortality of the mother trees. This finding aligns with previous studies on girdled *I. paraguariensis* and *Cedrela fissilis* Vell. mother trees, where researchers observed the resilience of the trees and subsequent recovery of the injured area over time (Wendling et al. 2013; Stuepp et al. 2016; Nascimento et al. 2018; Santos Junior et al.

FIGURE 4: Sprouting, number of sprouts and length of sprouts of *Moquiniastrum polymorphum* detached branches according to evaluation periods, in the municipality of Lages, Santa Catarina state, Brazil.



2021). These results are consistent with those obtained in this study, as the girdled *M. polymorphum* mother trees did not exhibit any symptoms of senescence during or after the evaluation periods.

2. Influence of individuals, disposition sense and time on the epicormic sprouting of detached branches

The results indicated that individual was a key factor for branch sprouting. Silva et al. (2021) also observed a similar response in detached branches of *Paratecoma peroba* (Record) Kuhlm, as substantial differences in sprouting were observed among mother trees, varying up to 40%. However, determining this factor is challenging, as it involves not only phenotypic characteristics but also hormones, dormant buds, and plant vigor. According to Hartmann et al. (2014), the percentage of sprouts in branches is possibly related to the endogenous levels of auxin and cytokinins present in the branches, regardless to the number of dormant buds.

The accumulation of higher quantities of cytokinins relative to auxins is a pivotal factor influencing the success of the sprouting technique. However, this may not exert a substantial influence on horizontally stored branches. The high percentage of sprouts in vertical branches can be explained by the ease of hormonal imbalance with an accumulation of cytokinins, as the branches were kept in their natural orientation (Taiz & Zeiger 2018; Hartmann et al. 2014), which may promote better development of dormant buds. In contrast, horizontal branches require rearrangement in the direction and orientation of these hormones for proper functioning, as there is a deviation

TABLE 4: Average* percentage survival, callus formation, rooting, sprouting and original leaves permanency of *Moquiniastrum polymorphum* canopy cuttings according to different individuals, in the municipality of Lages, Santa Catarina state, Brazil.

Individual	Survival (%)		Callus formation (%)		Rooting (%)		Sprouting (%)		Original leaves (%)	
3	8.0 ± 3.9†	d	6.8 ± 3.9	c	1.2 ± 0.7	c	2.5 ± 1.8	d	7.4 ± 3.7	C
4	3.7 ± 1.9	d	1.9 ± 1.3	c	3.1 ± 1.5	c	7.4 ± 4.8	d	2.5 ± 1.8	C
7	4.9 ± 1.8	d	4.3 ± 1.5	c	2.5 ± 1.6	c	2.5 ± 0.8	d	4.3 ± 1.8	C
50	2.5 ± 1.8	d	1.9 ± 1.3	c	1.2 ± 1.2	c	2.5 ± 1.8	d	1.9 ± 1.3	C
64	6.2 ± 3.1	d	5.6 ± 2.9	c	0.0	c	6.2 ± 3.1	d	1.2 ± 0.8	C
68	33.3 ± 4.1	c	33.3 ± 4.1	b	5.6 ± 2.5	c	29.6 ± 5.5	c	13.0 ± 3.4	C
70	1.2 ± 1.2	d	0.6 ± 0.6	c	0.6 ± 0.6	c	0.0	d	0.0	C
80	7.4 ± 3.7	d	7.4 ± 3.7	c	0.0	c	0.0	d	7.4 ± 3.7	C
84	11.1 ± 4.9	d	10.2 ± 4.9	c	8.3 ± 4.0	c	7.4 ± 3.4	d	4.6 ± 2.2	C
99	12.9 ± 6.0	d	11.1 ± 6.0	c	0.0	c	7.4 ± 4.7	d	5.6 ± 5.6	C
152	79.6 ± 13.6	a	75.9 ± 13.9	a	77.8 ± 13.1	a	79.6 ± 13.6	a	64.8 ± 13.9	A
165	16.7 ± 4.8	d	16.7 ± 4.8	c	9.3 ± 4.5	c	13.0 ± 5.3	d	9.3 ± 3.4	C
171	27.8 ± 4.8	c	26.0 ± 5.5	c	0.0	c	24.1 ± 6.0	c	5.6 ± 3.8	C
190	20.4 ± 10.2	c	20.4 ± 10.2	c	11.1 ± 5.0	c	16.0 ± 8.0	c	9.3 ± 7.4	C
212	12.9 ± 6.0	d	12.0 ± 5.8	c	0.0	c	12.0 ± 5.4	d	2.8 ± 1.9	C
213	57.4 ± 6.0	b	46.3 ± 5.3	b	57.4 ± 6.0	b	44.4 ± 5.7	b	42.6 ± 6.7	B
225	11.1 ± 5.6	d	8.6 ± 4.1	c	7.4 ± 4.2	c	6.8 ± 3.9	d	5.6 ± 4.1	C
Average	18.7 ± 2.3		17.0 ± 2.1		10.9 ± 2.2		15.3 ± 1.9		11.2 ± 2.2	

*Averages followed by the same letter do not differ according to the Scott-Knott average test; † Standard error was not added due to the low number of repetitions.

from the natural orientation (Taiz & Zeiger 2018).

The same pattern of sprout development over time observed in this study was also reported by Nascimento et al. (2018), who noted that horizontally positioned branches can have a longer lifespan but lower productivity. According to the same authors, horizontally oriented branches of *I. paraguariensis* can remain active for approximately 300 days. This same behavior was also observed by Silva et al. (2023) in *Drimys brasiliensis* Miers. In contrast, Wendling et al. (2013) reported that vertically oriented branches of *I. paraguariensis* can provide higher and faster sprout production but have a shorter lifespan. These findings indicate that the storage direction can directly influence sprout production and lifespan.

Despite substantial differences in sprouting variables between the two disposition senses, no branch mortality was observed. Additionally, no signs of degradation or senescence, such as excessive drying or changes in coloration, were detected.

3. Exploratory analyses of individuals and epicormic material

Given the detection of significant differences in cutting survival according to the individual, selection of individual may be crucial for this method of propagation. For *I. paraguariensis*, survival appeared to be closely associated with individuals, with variations of more than 50% observed among materials from the same population (Nascimento et al. 2022). The authors

TABLE 5: Survival, callus formation, rooting, sprouting and original leaves permanency of *Moquiniastrum polymorphum* cuttings according to the source of material, in the municipality of Lages, Santa Catarina state, Brazil

Material source	Survival (%)	Callus formation (%)	Rooting (%)	Sprouting (%)	Original leaves (%)
Canopy	18.7 ± 5.1†	17.0 ± 4.7	10.9 ± 5.3	15.3 ± 4.0	11.2 ± 3.6
Branches	25.8 ± 5.4	17.5 ± 5.2	11.2 ± 4.7	12.2 ± 2.8	16.3 ± 4.5
Average	22.2 ± 3.7	17.3 ± 2.8	11.0 ± 3.4	13.8 ± 2.3	13.8 ± 2.4

† Standard error was not added due to the low number of repetitions.

TABLE 6: Survival, callus formation, rooting, sprouting and original leaves permanency of *Moquiniastrum polymorphum* canopy cuttings according to different individuals in two rooting environments, in the municipality of Lages, Santa Catarina state, Brazil.

	Individual						
	3	**7**	**50**	**64**	**80**	**190**	**Average***
Environment	Survival (%)						
MT[1]	20.4 ± 3.4†	5.6 ± 3.8	5.6 ± 2.5	7.7 ± 2.3	7.4 ± 3.7	9.3 ± 3.4	8.6 ± 1.5
GWIN[2]	9.3 ± 3.4	13.0 ± 3.4	0.0	0.0	1.9 ± 1.9	7.4 ± 4.7	5.2 ± 1.3
Average*	14.8 ± 2.8 a	9.3 ± 2.7 a	2.8 ± 1.5 b	1.9 ± 1.2 b	4.6 ± 2.1 b	8.3 ± 2.8 a	6.9 ± 0.9
Environment	Callus formation (%)						
MT	18.5 ± 2.3	5.6 ± 3.8	3.7 ± 2.5	3.7 ± 2.3	7.4 ± 3.7	9.3 ± 3.4	8.0 ± 1.4
GWIN	9.3 ± 3.4	13.0 ± 3.4	0.0	0.0	1.9 ± 1.9	7.4 ± 4.7	5.2 ± 1.4
Average*	13.9 ± 2.4 a	9.3 ± 2.7 a	1.9 ± 1.2 b	1.9 ± 1.2 b	4.6 ± 2.1 b	8.3 ± 2.8 a	6.6 ± 0.8
Environment	Rooting (%)						
MT[1]	3.7 ± 2.3	0.0	3.7 ± 2.3	0.0	0.0	7.4 ± 2.3	2.5 ± 0.8
GWIN[2]	5.6 ± 3.8	0.0	0.0	0.0	1.9 ± 1.9	5.6 ± 3.8	2.2 ± 1.0
Average*	4.6 ± 2.1 a	0.0 b	1.9 ± 1.2 b	0.0 b	0.9 ± 0.9 b	6.5 ± 2.1 a	2.3 ± 0.5
Environment	Sprouting (%)						
MT[1]	7.4 ± 3.7	3.7 ± 2.3	5.6 ± 2.5	3.7 ± 2.3	0.0	7.4 ± 3.7	4.6 ± 1.1
GWIN[2]	5.6 ± 2.5	11.1 ± 2.9	0.0	0.0	1.9 ± 1.9	7.4 ± 4.7	4.3 ± 1.2
Average*	6.5 ± 2.1 a	7.4 ± 2.1 a	2.8 ± 1.5 b	1.9 ± 1.2 b	0.9 ± 0.9 b	7.4 ± 2.8 a	4.5 ± 0.7
Environment	Original leaves (%)						
MT[1]	18.5 ± 4.7	3.7 ± 2.3	3.7 ± 2.3	0.0	7.4 ± 3.7	1.9 ± 1.9	5.9 ± 1.5 a
GWIN[2]	5.6 ± 3.8	1.9 ± 1.9	0.0	0.0	0.0	0.0	1.2 ± 0.8 b
Average*	12.0 ± 3.5 a	2.8 ± 1.5 b	1.9 ± 1.2 b	0.0 b	3.7 ± 2.1 b	0.9 ± 0.9 b	3.5 ± 0.7

[1] MT: Mini Tunnel; GWIN: [2] Greenhouse With Intermittent Nebulization
*Averages followed by the same letter do not differ according to the Scott-Knott average test; †Standard error was not added due to the low number of repetitions.

also suggest that each individual, in addition to the physiological conditions of the mother trees, requires specific environmental conditions for rooting in order to enhance survival. Similar conditions may also apply to *M. polymorphum*, as variations in survival exceeding 75% were observed among individuals. It is possible that the rooting environmental conditions were optimal for individual 152, explaining its overall superior development.

Furthermore, survival rates can be improved for *I. paraguariensis* and *D. brasiliensis* cuttings produced from epicormic sprouts, reaching levels higher than 75% and 68%, respectively (Stuepp et al. 2017; Silva et al. 2023). However, in the case of *M. polymorphum*, cuttings produced from epicormic sprouts from detached branches did not exhibit improved survival rates. Although detached branches are efficient in producing epicormic sprouts, their vigor, i.e., ontogenetic age, is still influenced by their collection height (Hartmann et al. 2014). Since the *M. polymorphum* branches were collected at heights above 1.5 m, the ontogenetic factor may have played a significant role, and the epicormic sprouts produced could be considered mature.

In this context, studies by Wendling et al. (2013), Stuepp et al. (2017), and Nascimento et al. (2018) have mentioned that the presence of callus is common in mature propagules of *I. paraguariensis* that have not undergone any rejuvenation technique. However, high maturity does not necessarily mean an inability to root. Callus formation indicates the initiation of cell division and differentiation (Hartmann et al. 2014), and under optimal conditions, there is a high likelihood of rooting the propagule (Stuepp et al. 2017; Nascimento et al. 2018). This may also apply to *M. polymorphum* cuttings, as most of the rooted material exhibited callus formation regardless of individuals or material origin.

According to Nogueira et al. (2017) and Belniaki et al. (2018), the presence of leaves in cuttings of plant species promotes greater root development, with more roots and longer lengths. This relationship can be explained by the production of phenolic compounds, such as auxin, by the aerial part of the plant (Vignolo et al. 2014). Therefore, for some species, the presence of leaves is a crucial factor for successful rooting during the cutting process (Nogueira et al. 2017). The best-rooted *M. polymorphum* individuals exhibited high percentages of new sprouts, original leaves, or both, corroborating the existing literature.

The differences observed among individuals in the rooting of cuttings indicate that this process may not be exclusively inherent to the species. Fachinello et al. (2005) mention that the ability of a cutting to form roots can be greatly influenced by the clone, due to its correlation with endogenous concentrations of essential substances in root induction, which vary with the origin of the genetic material. Wendling et al. (2017) highlight that, for the *Araucaria angustifolia* (Bertol.) Kuntze, the rooting process of cuttings is linked to the genotype, as the rooting capacity of the cuttings is impacted by the provenance of the genetic material. In *M. polymorphum*, the rooting potential of cuttings also showed a high discrepancy among the studied individuals. Despite the absence of previous studies on the vegetative propagation of this specific species, it can be inferred that the observed variations are primarily of genetic nature. Since the individuals are derived from the same population, it is likely that the differences observed are a result of individual genetic variations. In other words, different individuals within the population may exhibit distinct responses in terms of their rooting capacity through vegetative propagation (Franzon et al. 2010).

4. Cutting according to adventitious rooting environments and individuals

As the rooting environments did not make significant differences for most of the variables, it is reasonable to assume that the previously discussed conditions in this paper remain the most relevant factors. Alternatively, the lack of significant differences suggests that further research is necessary to improve the analysis of rooting in *M. polymorphum* cuttings.

However, it should be noted that the retention of original leaves was nearly five times higher in the MT environment compared to the GWIN environment, although it remained below 6% in both environments. It is possible that the higher and more stable relative humidity provided by the MT environment reduced stress levels for the original leaves, resulting in reduced senescence. Furthermore, Brondani et al. (2008) mention that the presence of leaves in cuttings is primarily determined by the genetics of the mother plant, but the conditions of the rooting environment can either promote or hinder leaf retention.

The relationship between survival and rooting rates in different rooting environments may appear perplexing for certain individuals. For instance, when examining individuals 3 and 80, it is evident that although survival was considerably higher in the MT environment, rooting was lower or did not occur in this particular setting. However, this pattern was not consistently observed for individuals 190, which displayed similar survival and rooting ratios between both environments. A similar phenomenon was also noted by Nascimento et al. (2020; 2022) in their study on *I. paraguariensis* cuttings. The authors observed that, in general, survival rates were approximately 20% higher in the MT environment, while rooting rates were nearly two times more successful in the GWIN environment. However, this pattern did not hold true for all individuals.

Furthermore, the authors suggested that for *I. paraguariensis*, high humidity levels favored higher survival rates but hindered rooting, while the opposite could also be true. This relationship appears to be applicable to *M. polymorphum* cuttings as well. The higher humidity provided by the MT environment may have contributed to maintaining survival rates, while the lower humidity in the GWIN environment may have stimulated rooting. Further analyses in different rooting environments under singular climatic conditions are necessary to assess this hypothesis.

Conclusions

In addressing the questions raised in the introduction, the following key findings should be emphasised:

(1) There are distinct responses in terms of vegetative rescue and propagation among different individuals of *M. polymorphum* within the same population;

(2) No significant differences were found in the rooting of cuttings between canopy and epicormic material; and

(3) Specific rooting environments do not have a significant impact on the rooting of cuttings.

The vegetative propagation of *M. polymorphum* is feasible using canopy material, which simplifies the process of vegetative rescue. Simple rooting environments with minimal control of climatic conditions can be utilised for successful rooting of the cuttings.

Considering the lack of existing studies on vegetative rescue and propagation of *M. polymorphum,* it is recommended that further research be conducted in this area, exploring new variables, and conducting more comprehensive analyses to enhance our understanding of these processes. New tests could consider more populations, alternative techniques for material reinvigoration, and different rooting environments.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

ACSS established the hypothesis, performed data collection, evaluations and analyses, besides writing results and discussion. BN conducted a review of the subject and performed the writing of methodology, results, discussion and translated the manuscript. BJSC performed material collection and processing. GNS performed material collection and processing. MCN, LMO, AM and MOP perfomed a review of the manuscript and supervised its general development, besides approving the final version.

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