

Effects of the phenological stage, cutting type and plant growth regulators on propagation with stem cuttings of *Poiretia latifolia* Vogel, a Brazilian native medicinal plant

Efectos de la etapa fenológica, tipo de corte y reguladores del crecimiento vegetal en la propagación por corte del tallo de *Poiretia latifolia* Vogel, una planta medicinal nativa de Brasil



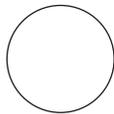
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***Poiretia latifolia* cutting.**

Photo: R. Verdi.

ABSTRACT

Poiretia latifolia Vogel has bioactive potential that includes antibiotic properties for humans. The aim of this research was to develop an efficient method for the vegetative propagation of *P. latifolia* with cuttings to aid the selection and multiplication of interesting genotypes. In the first experiment, the cuttings were collected in vegetative and reproductive stages and treated with six plant growth regulators and distilled water. In the second experiment, the treatments consisted of cuttings from the apical, median, and basal branch segments. The treatment that immersed cuttings in 4,000 mg L⁻¹ of IBA provided a greater number and length of the roots, while the high dilution of *Calcareo phosphorica* at 20CH produced longer branches. The cuttings from the middle and basal part of the branches were more viable. 79.17% rooting of the cuttings was achieved with *Calcareo phosphorica* 20CH. The vegetative propagation employed in this study is a feasible option for the domestication, preservation, and cultivation of *Poiretia latifolia*.



Additional keywords: auxin; high dilution; erva-de-touro; domestication.

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RESUMEN

Poiretia latifolia Vogel tiene un potencial bioactivo que incluye propiedades antibióticas para usos humanos. El objetivo de esta investigación fue desarrollar un método eficiente para la propagación vegetativa de *P. latifolia* utilizando esquejes como ayuda para la selección y multiplicación de genotipos interesantes. En el primer experimento, los esquejes se recolectaron en etapas vegetativas y reproductivas y se trataron con seis reguladores del crecimiento vegetal y agua destilada. En el segundo experimento, los tratamientos consistieron en estacas de rama apical, mediana y basal. El tratamiento con la inmersión de esquejes en 4,000 mg L⁻¹ de IBA proporcionó un mayor número y longitud de las raíces, mientras que la alta dilución de *Calcareea phosphorica* a 20CH produjo ramas más largas. Se encontró que los esquejes de la parte media y basal de la rama eran más viables. Se logró el 79,17% de los esquejes de enraizamiento utilizando *Calcareea phosphorica* 20CH. La propagación vegetativa empleada en este trabajo es una opción factible para la domesticación, preservación y cultivo de *Poiretia latifolia*.

Palabras clave adicionales: auxina; alta dilución; erva-de-touro; domesticación.

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INTRODUCTION

Poiretia latifolia Vogel (Fabaceae), popularly known as “erva-de-touro”, is traditionally used in the treatment of stomach and urinary problems, for flavoring traditional yerba mate and as an aphrodisiac (Müller, 1984; Amorim and Boff, 2009). Nanoemulsion, based on its essential oil, has potential as an anti-dermatophyte and anti-inflammatory agent (Porto *et al.*, 2010; Pérez-Zamora *et al.*, 2016; Paim *et al.*, 2018). In addition, *Poiretia latifolia* could be an alternative income for farmers since its essential oil is rich in components that are important to the flavour and fragrance industry, such as carvone, dihydrocarvone, and limonene (Porto *et al.*, 2010; Fernandes and Boff, 2017).

Poiretia latifolia naturally occurs on the South Plateau of Santa Catarina, Southern Brazil in the Ombrofila Mista Forest (Fernandes and Boff, 2017). The plant diversity of this region has the potential for numerous uses, for example, grazing, food, medicinal and cosmetic uses. However, this potential has been threatened by anthropogenic activities, such as cattle, fruit orchards, intensive cultivation of soybean and corn, and reforestation with *Pinus* sp. and *Eucalyptus* sp. (Pereira *et al.*, 2006; Zank and Hanazaki, 2012). In order to strengthen plant diversity and enhance its potential, the most appropriate propagation technique for plants such as *Poiretia latifolia* is essential (Nogueira and Nogueira-Filho, 2011; Sarasan *et al.*, 2011; Vodouhè *et al.*, 2011).

Poiretia latifolia seeds have a germination rate above 80%; however, its production is severely damaged by insects, which destroy 54% of its flowers and 88% of its seeds (Maass *et al.*, 2018). Vegetative propagation could overcome this problem and even facilitate the selection of the best plant characteristics (Rasmussen *et al.*, 2015; Kaviani and Nagahdar, 2017; Wetzstein *et al.*, 2018). However, vegetative propagation knowledge for *P. latifolia* is limited and unsuccessful (Teixeira *et al.* 2011; Amorim *et al.*, 2012). The success of this technique relies on adventitious rooting, which is influenced by several factors, such as plant phenological stage, cutting type and levels of inhibitors and/or promoters of plant growth in the propagule (Guo *et al.*, 2009; Zerche and Druège, 2009; Ludwig-Müller, 2011; Pacurar *et al.*, 2014; Pigatto *et al.*, 2018).

Exogenous plant growth regulators are widely used in vegetative propagation and can be synthetic or natural. However, when working with medicinal plants, natural alternatives are preferable. Yamashita *et al.* (2017) found that *Cyperus rotundus* extract (natural) and indol-3-butiric acid - IBA (synthetic) improved the rooting of *Myrciaria cauliflora* and *Psidium guajava* cuttings. High dilutions are another alternative that aid production, minimizing residues and reducing costs (Moreno, 2017). The homeopathic preparations *Phosphorus* 3CH and *Kali muriaticum* 12CH, selected based on the nutrients commonly used in plant fertilization, promoted increased root growth

in eucalyptus seedlings. (Moraes *et al.*, 2018). Cassol *et al.* (2017) also reported that the use of IBA and *Arnica montana* 12 CH stimulated the rooting of *Cuphea gracilis*.

The objective of this study was to evaluate the influence of phenological stages, segments of the branch and plant growth regulators on the vegetative propagation of *P. latifolia* using stem cutting.

MATERIALS AND METHODS

Collection and conduction locations

This study was conducted at the Lages Experimental Station of the EPAGRI (Agricultural Research and Rural Extension Agency of Santa Catarina State, Brazil). The vegetative material used in the experiments was collected from a natural population of *Poiretia latifolia* in the region of Vacas Gordas, district of Capão Alto, Santa Catarina (27°58'33.1" S and 50°30'26.5" W). Immediately after the sampling, the herbaceous shoots were immersed in water and transported in polystyrene boxes to avoid dehydration of the vegetal material.

Experiment design

In the first experiment, seven treatments with plant growth regulators were evaluated: immersion of the base of the cuttings for 20 s in four concentrations of indole-3-butyric acid (IBA) (1,000, 2,000, 3,000, and 4,000 mg L⁻¹); aqueous extract of *Cyperus rotundus*, 10% concentration; high dilution of *Calcarea phosphorica* at 20CH (CH = hahnemannian centesimal dilution order); and pure water (control). Each treatment had three replicates of 20 cuttings in a completely randomized block design. The experiment was carried out in both phenological stages of *P. latifolia*: vegetative (14 December, 2016) and reproductive (18 January, 2017). In total, 840 cuttings were used in the experiment.

In the second experiment, the treatments consisted of the cutting type in terms of the segment of the *P. latifolia* branch, divided into the basal, median, and apical thirds. Each treatment had four replicates of 20 cuttings in a randomized block design, totaling 240 cuttings in the experiment. The cuttings were collected at the reproductive stage (22 February, 2017).

Obtaining and applying plant growth regulators

The IBA was dissolved in 1N NaOH and adjusted to the concentrations with distilled water. The aqueous extract of *Cyperus rotundus* was obtained by blending 100 g of fresh plant bulbs in 1,000 mL of pure water in a blender for 10 min. The high dilution of *Calcarea phosphorica* was obtained from a pharmacy, and the last two levels of dynamization were made with pure water, following the methodology described in the Brazilian Homeopathic Pharmacopoeia (2011). The plant growth regulators were applied with immersion for 20 s with 1/3 of the base of the cuttings in the solution. The high dilution of *Calcarea phosphorica* at 20CH was applied to the aerial part of the cuttings with spraying twice a week at a volume of 50 mL per replicate until the end of the experiment. During this period, the other treatments received pure water.

Cutting preparation and planting

The herbaceous *P. latifolia* shoots were segmented into cuttings with two lateral buds. In the first experiment, all branch segments were used and placed randomly inside the blocks, producing cuttings with varied lengths and diameters. In the second experiment, the diameter and length of the cuttings varied according to the characteristics of the segment evaluated: basal, median, or apical.

The preparation of the cuttings for all experiments and treatments followed the protocol described by Bettoni *et al.* (2014b). At the base of the cuttings, a transverse cut was made, with 20% of the total length of the cutting remaining below the lateral bud. At the top end of the cutting, an angle cut was made, with 30% of the total length of the cutting remaining above the lateral bud. Afterwards, at the base of the cuttings, two longitudinal lesions were made, with approximately 30% of the total length of the cuttings. A leaflet was maintained on each cutting.

After preparation, the cuttings were transplanted to Styrofoam honeycomb trays with 60 cells with sand and vermiculite substrate (1:1, v/v). The trays were arranged in plastic boxes (60 x 40 x 20 cm) and covered with a transparent plastic film to maintain moisture saturation. They were watered with spraying twice per day and kept in a greenhouse at 25°C. No control for pests and diseases was carried out.

Statistical analysis and assessment

Survival rate, rooting rate, shoot sprouting rate, number of roots and shoots, and length of the four larger roots and the two larger shoots were assessed 35 d after planting the cuttings. In order to analyze survival, rooting, and sprouting, generalized linear models were used with the binomial model or the binomial model with a dispersion parameter (quasi-binomial), both considering the logit link function. The means were compared with confidence intervals (95%) for Tukey contrasts of the linear predictors of the fitted model. All analyses were performed using the R environment v 3.2.1 (The R Foundation, 2015).

RESULTS AND DISCUSSION

The joint analysis of the experiment with plant growth regulators in the vegetative and reproductive phases of *P. latifolia* plants showed the simple effect of the treatments (Tab. 1), meaning that the plant growth regulators performed similarly in the two phenological stages: vegetative and reproductive.

The phenological stage of the *P. latifolia* plants influenced the cutting viability through the rate of surviving, rooting, and sprouted cuttings; the vegetative stage was the lowest (Tab. 2). The plant growth regulators did not increase the cuttings viability. Cuttings

Table 1. Descriptive levels (p-values) of the joint analysis of propagation with *Poiretia latifolia* cuttings and plant growth regulators in the vegetative and reproductive stages, Lages, SC, 2018.

Factors	SURV.	R	R/C	LR	S	S/C	LS
PE	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0430	0.3806
PE/Block	0.0371	0.0230	0.1738	0.0085	0.0352	0.7835	0.1011
GR	0.0417	0.0293	<0.0001	<0.0001	0.0369	0.0438	0.0069
GR*PE	0.0724	0.0699	0.6407	0.5178	0.1897	0.9826	0.2279

PE = phenological stage, GR = growth regulators, SURV. = survival rate of cuttings, R = rooting rate, R/C = roots per cutting, LR = mean length of the four largest roots, S = sprouting rate, S/C = shoots per cuttings, LS = mean length of the two largest shoots.

Table 2. Viability of *Poiretia latifolia* cuttings collected in different phenological stages, treated with plant growth regulators and from different sections of the branch. Lages, SC, 2018.

Treatments	Survival (%)	Rooting (%)	Sprouting (%)
Phenological stages of plant collection ¹			
Vegetative	53.81 ± 3.16 b	55.71 ± 3.01 b	49.52 ± 3.25 b
Reproductive	81.90 ± 2.22 a	82.62 ± 2.09 a	70.00 ± 2.50 a
Plant growth regulators ¹			
Control	68.33 ± 5.43 ab	68.33 ± 5.11 ab	60.83 ± 3.52 ab
1,000 mg L ⁻¹ IBA	57.50 ± 9.20 b	58.33 ± 8.63 b	47.67 ± 8.82 b
2,000 mg L ⁻¹ IBA	66.67 ± 8.72 ab	70.00 ± 8.66 ab	63.33 ± 8.23 ab
3,000 mg L ⁻¹ IBA	64.17 ± 7.46 ab	67.50 ± 6.42 ab	60.83 ± 8.41 ab
4,000 mg L ⁻¹ IBA	72.50 ± 6.68 ab	74.17 ± 7.00 ab	60.00 ± 4.83 ab
<i>C. rotundus</i>	66.67 ± 10.46 ab	66.67 ± 9.80 b	64.17 ± 10.36 ab
<i>Calc. phos.</i> 20CH	79.17 ± 5.54 a	79.17 ± 5.39 a	72.50 ± 4.23 a
Sections of the branch ²			
Apical	2.50 ± 1.44 b	2.50 ± 1.44 b	5.00 ± 0.00 c
Median	16.25 ± 1.25 a	32.50 ± 2.50 a	17.50 ± 1.44 a
Basal	12.50 ± 2.50 a	46.25 ± 2.39 a	12.50 ± 2.50 b

Control = distilled water, *C. rotundus* = aqueous extract of *Cyperus rotundus*, *Calc. phos.* 20CH = High dilution of *Calcareo phosphorica* at 20CH (CH = Hahnemannian centesimal dilution order). Different letters in the columns present a statistical difference between treatments, Tukey's contrast ($P \leq 0.05$). Means ± standard error; ¹ Trials analyzed together; ² Independent trial.

from the medial and basal branch sections performed better than those from the apical section. The sprouting rate was still better for the basal section than for the median section (Tab. 2).

The vegetative stage, contrary to that found for viability (Tab. 2), increased the number of roots, average length of the four larger roots, number of shoots, and average length of the two bigger shoots (Tab. 3).

The IBA concentrations 3,000 and 4,000 mg L⁻¹ had higher root numbers than the treatments without synthetic auxin applications. IBA at 4,000 mg L⁻¹ was superior to 2,000 mg L⁻¹ but did not differ from 3,000 mg L⁻¹ for the length of the four larger roots (Tab. 3). Increases in the IBA concentration increased the number of roots per cutting and the average length of the four larger roots, which had an inverse relationship with the shoot number and the length of the two larger shoots (Tab. 3).

The growth regulators did not influence the number of shoots per cutting. However, *Calcarea phosphorica* 20CH increased the length of the two larger shoots of the cuttings but did not differ from the IBA concentrations 1,000 and 2,000 mg L⁻¹ (Tab. 3). The different

branch sections did not differ in the quality of the viable *P. latifolia* cuttings (Tab. 3).

During the plant collection, the branches were more lignified in the reproductive stage than in the vegetative stage. Cuttings from older plants that are more lignified, besides having more nutrient reserves because of the tissue consistency, are more resilient to environmental stresses, such as temperature and humidity (Villa *et al.*, 2017). This may explain the better cutting viability for the reproductive stage and the medial and basal branch segments of *P. latifolia* plants (Tab. 2).

However, the vegetative stage had better cutting quality than the reproductive stage, the opposite of that found for cutting viability. According to Maia *et al.* (2008), more lignified cuttings may impede rooting, observing better rooting in less lignified cuttings of the apical segment of *Hyptis suaveolens* branches. Even though lower nutritional reserves and less resistance to stresses impede cutting viability, cuttings from the vegetative stage may have a better hormonal balance, improving rooting quality in surviving cuttings (Ludwig-Müller, 2011; Pacurar *et al.*, 2014).

Table 3. Quality of viable *Poiretia latifolia* cuttings collected in different phenological stages, treated with plant growth regulators and from different sections of the branch. Lages, SC, 2018.

Treatments	R/C (n°)	LR (cm)	S/C (n°)	LS (cm)
Phenological stages of plant collection¹				
Vegetative	21.46±0.82 a	18.39±0.65 a	1.35±0.06 a	8.18±0.46 a
Reproductive	16.77±0.63 b	15.67±0.49 b	1.19±0.04 b	7.78±0.32 b
Plant growth regulators¹				
Control	16.97±1.35 bc	15.35±1.22 b	1.25±0.10 ab	7.59±0.43 b
1,000 mg L ⁻¹ IBA	19.58±1.24 abc	17.46±1.14 ab	1.27±0.11 ab	8.04±0.71 ab
2,000 mg L ⁻¹ IBA	20.90±1.26 ab	17.57±0.41 b	1.31±0.08 ab	8.27±0.79 ab
3,000 mg L ⁻¹ IBA	22.47±1.54 a	17.66±0.94 ab	1.07±0.09 b	6.81±0.67 b
4,000 mg L ⁻¹ IBA	21.63±1.42 a	20.46±1.07 a	1.10±0.06 ab	6.79±0.52 b
<i>C. rotundus</i>	15.83±1.81 c	14.16±1.37 b	1.40±0.07 ab	8.13±0.68 b
<i>Calc. phos.</i> 20CH	16.42±1.14 c	16.58±0.61 b	1.52±0.11 a	10.26±0.67 a
Sections of the branch²				
Apical	4.00±2.00 a	11.90±9.10 a	1.00±0.00 a	3.00±2.00 a
Median	7.58±0.64 a	13.12±1.76 a	1.17±0.10 a	5.04±0.83 a
Basal	7.37±1.40 a	9.63±1.07 a	1.12±0.12 a	3.12±1.16 a

Control = distilled water, *C. rotundus* = aqueous extract of *Cyperus rotundus*, *Calc. phos.* 20CH = high dilution of *Calcarea phosphorica* at 20CH (CH = Hahnemannian centesimal dilution order), R/C = roots per cutting, LR = mean length of the four larger roots, S/C = shoots per cuttings, LS = mean length of the two larger shoots. Different letters in the columns present a statistical difference between treatments, Tukey's contrast ($P \leq 0.05$). Means ± standard error; ¹ Trials analyzed together; ² Independent trial.

The IBA effects on root quality in this study were also observed by Bettoni *et al.* (2014a) and Amorim *et al.* (2012), who observed that, although IBA did not change the cutting viability of grapevine rootstocks and *Poiretia latifolia* cuttings, it improved the quality of the surviving cuttings. Amorim *et al.* (2012) suggested that studies on higher IBA concentrations and other rooting inducers. However, the application of IBA or increases its doses does not necessarily promote the quality of cuttings. Benzylaminopurine (BAP) concentrations from 0.2 to 3.0 mg L⁻¹ did not enhance *Poiretia latifolia* vegetative propagation, and although there was not a statistical difference, the treatment with 2,000 mg L⁻¹ of AIB promoted a better rooting rate than the treatment with 4,000 mg L⁻¹ (Teixeira *et al.*, 2011).

The high dilution of *Calcarea phosphorica* at 20CH promoted shoot growth and did not interfere with root quality (Tab. 3). *Calcarea phosphorica*, when applied at a young age, strengthens plants, improving nutrient absorption and resilience when suffering a hydric stress (Moreno, 2017). However, choosing a high dilution that provides the desired effect is somewhat complex and depends on several factors, such as the preparation method and the potency, application, concentration and nature of the preparation (Hanif and Dawar, 2015; Giesel *et al.*, 2017). Giesel *et al.* (2017) reported that both the potency and the dynamization method influence the effect of a high dilution on the foraging of leaf-cutting ants (*Acromyrmex laticeps* Emery). According to Broetto *et al.* (2011), the quality of seedlings is associated with the development of both the aerial part and roots. Thus, more studies are needed to achieve results similar to that of the study by Pulido *et al.* (2014), who was able to stimulate both aerial growth and cabbage roots. The homeopathic preparations *Phosphorus* 3CH and *Kali muriaticum* 12CH, selected based on the nutrients commonly used in plant fertilization, promoted increased root growth in eucalyptus seedlings (Moraes *et al.*, 2018). Cassol *et al.* (2017) also reported that the use of IBA and *Arnica montana* 12 CH, usually used to help an organism cope with traumatic events such the cutting process, stimulated the rooting of *Cuphea gracilis*.

The results obtained from the *P. latifolia* cuttings treated with *C. rotundus* extracts were similar to those of Yamashita *et al.* (2017), who tested five concentrations of the extract (0, 25, 50, 75, and 100%) and did not observe a difference in the number of roots in *Myrciaria cauliflora* and *Psidium guajava*. The authors

suggested that the auxin concentration in the aqueous extract of *C. rotundus* is not sufficient to increase the number of roots. Dias *et al.* (2012) also warned that, depending on the cutting immersion time, the extract may have toxicity effects.

Even though there was no increase in the rooting rate with the plant growth regulator treatments, the application of 4,000 mg L⁻¹ of IBA provided better development of the root system in the *P. latifolia* cuttings. The high dilution of *Calcarea phosphorica* at 20CH provided better development of the aerial part of the *P. latifolia* cuttings. The methodology for the cutting collection and preparation, as well as the conduction period of the rooting, provided a higher rooting rate (79.17%) than in previous studies (around 10 and 40%) (Amorim *et al.*, 2012; Teixeira *et al.*, 2011).

CONCLUSION

Calcarea phosphorica achieved a rooting rate of 79.17%. The high dilution improved the aerial part of the *P. latifolia* cuttings, while the AIB enhanced the rooting quality. More studies on these two plant growth regulators, both individually and combined, should be carried out to improve the vegetative propagation of this species. More lignified *Poiretia latifolia* cuttings are more viable because of the phenological stage and the position of the branch segment. The vegetative propagation used in this study is a feasible option for the domestication, preservation, and cultivation of *Poiretia latifolia*.

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Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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