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Sexual and asexual propagation of *Baccharis dracunculifolia* DC., a dioecious medicinal Brazilian shrub

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ABSTRACT. *Baccharis dracunculifolia* DC. is a pioneer plant native from Brazil that has great importance due to its secondary metabolites (essential oil and Brazilian green propolis) and its potential in the recovery of degraded areas. The main goal of this study was to evaluate the propagation techniques of *B. dracunculifolia* by sexual reproduction and asexual propagation of male and female stem cuttings. For reproduction, female individuals from a natural population were periodically monitored for their reproductive development and their diaspores were collected to evaluate the maximum dry mass accumulation to determine physiological maturity. The germination test was performed by testing four temperatures (20, 25, 30, and 20–30°C), in addition to the germination speed index (GSI) and first count. For vegetative propagation, stem cuttings (8 cm) of male and female individuals were treated with an indole butyric acid (IBA) solution at 0, 1,500, 3,000, 4,500, and 6,000 mg L^{-1}. The cuttings were evaluated for mortality, survival, rooting, sprouting, leaf retention, callogenesis, number of roots, and average length of roots (cm) after 120 days. The physiological maturity of diaspores occurred at 40 days after anthesis, which was the best time for collection in the field. The temperatures of 25 or 20–30°C should be used in the germination tests of the species. The first count was identified four days after sowing and the last count after 11 days. The rooting of *B. dracunculifolia* cuttings is very low. Sex did not influence the evaluated parameters, but increasing IBA doses positively influenced rooting, number of roots, and average length of roots and negatively influenced calluses formation.

Keywords: alecrim-do-campo; asteraceae; indole butyric acid; seed quality; stem-cutting rooting; plant reproduction.

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Introduction

Baccharis dracunculifolia DC. (Asteraceae) is a medicinal species native from Brazil and widely distributed in South America. The plant is a perennial woody shrub (2–3 meters high), dioecious, with flowers aggregated into inflorescences (capitulum) and fruits consisting of glabrous achenes with 1.5 mm in length (Barroso & Bueno, 2002). *B. dracunculifolia* is well known as *Apis mellifera* main bee pasture to produce Brazilian green propolis, a product with numerous beneficial properties for human health (Sforcin et al., 2012; Bankova et al., 2019) and potential use as antimicrobial and antioxidant activity (Veiga et al., 2017). Besides that, the plant has an essential oil rich in pharmacological and biological activities (Chan, Tan, Chan, Lee, & Goh, 2016). Among the plants of the genus *Baccharis* found in Brazil, *B. dracunculifolia* is the species with the most characteristics of anthropic pioneer (Barroso & Bueno, 2002) and has a high rate of natural regeneration, which emphasizes its ecological importance in the recovery of degraded areas (Campos & Martins, 2016).

Despite the ecological and economic importance of this plant, information about the propagation of the species is limited. The natural method of reproduction of *B. dracunculifolia* is by diaspores, but only a few studies on germination are available in the literature (Gomes & Fernandes, 2002; Manfreda, Alcaraz, & Scaramuzzino, 2020), and important information such as the optimal point of harvest, which is an essential factor for obtaining high-quality plant material, is absent. The optimal point of seed harvesting is established by the point of physiological maturity, identified by the maximum dry mass accumulation (Rajou et al., 2012).

Considering that most green propolis beekeepers in Brazil produce their own seedlings of *B. dracunculifolia* by diaspores, the lack of information about its harvesting time may reduce the success of quality seedling production. In addition, the definition of the ideal temperature for the species germination through parameters such as percentage of germination and vigor tests can also assist in determining the best time for sowing, leading to economic impacts to beekeepers and medicinal plant producers.

Currently, there are no recommendations for the vegetative propagation of *B. dracunculifolia*, but the vegetative propagation by cuttings for the species *Baccharis trimera*, *Baccharis articulata*, and *Baccharis stenocephala* has been effective (Bona, Biasi, Zanette, & Nakashima, 2005). The vegetative propagation by cuttings is the most widely used reproduction method in the commercial production of various medicinal, fruit, and ornamental crops and is characterized by the maintenance of genetic stability (Hartmann, Kester, Davies, & Geneve, 2011). The vegetative propagation of medicinal plants such as *B. dracunculifolia* can mean maintaining uniformity for the production of secondary metabolites, which can increase the quality of their final products, such as green propolis and essential oils.

Dioecious species, such as *B. dracunculifolia*, may present morphological and physiological differences between male and female plants due to resource allocation strategies developed to maximize reproductive success (Besten et al., 2013; Robinson et al., 2014). Previous studies on the vegetative propagation of dioecious species such as *Araucaria angustifolia* have identified the influence of the sex of plants on the rooting of stem cuttings (Wendling, Stuepp, & Zuffellato-Ribas, 2016). It may be related to the phenolic contents in the plant, which can vary between male and female plants, as triphenols, o-diphenols, and m-diphenols have been reported to reduce IAA decarboxylation and enhance rooting (De Klerk, Guan, Huisman, & Marinova, 2011).

Auxins are important phytohormones that regulate adventitious root induction (Steffens & Rasmussen, 2016). The best known endogenous auxins are indole-acetic acid (IAA) and indole-butyric acid (IBA), and the difference between them is related to the stability because IAA is relatively labile and IBA has a lower instability, which induces adventitious roots and promotes lower sensitivity to oxidation, being also the most applied auxin for root induction in woody species (Stuepp, Wendling, Trueman, Koehler, & Zuffellato-Ribas, 2017).

In this sense, the main goal of this study was to evaluate the propagation techniques of *B. dracunculifolia* by sexual reproduction and asexual propagation using stem cuttings.

Material and methods

Sexual propagation

Monitoring of maturation

Plants of *B. dracunculifolia* were monitored between January and April 2017 during the flowering and seed production of the species. These plants were present in a natural population located in southern Brazil in the Araucaria Atlantic Forest in the municipality of Palmeira, Paraná State, Brazil (25°19′80″ S and 49°48′35″ W, with an altitude of 1,027 meters). The regional climate is classified as Cfb (Alvares et al., 2013). The species was confirmed from herbarium analyses in the Municipal Botanical Museum of Curitiba – MBM Herbarium, with the voucher specimen deposited under the number MBM-370.693. The registration number of the species in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) is AC74344.

Ten branches were marked and monitored weekly for each individual of the 20 female parent trees selected at the site until 50% of the inflorescences were at anthesis. Subsequently, field observations were reduced to twice a week to monitor seed formation. The diaspores from this material (achene + pappus) were mixed, the seeds were separated through sieve friction using a circular sieve with openings of 1.8 and 1.6 mm in diameter, and then homogenized by the manual method (Brasil, 2009). The diaspores were separated for the maturation study when they presented a complete formation, and the hardening of the integument was identified.

Dry mass analysis

Twenty diaspores were collected per marked branch from all the selected parent trees in each performed collection, i.e., at 19, 26, 29, 33, 36, and 40 days after anthesis (DAA). Four replicates of 200 diaspores were weighed using an analytical balance (0.001 g) and packed in Kraft paper bags. The bags containing the diaspores were dried in a forced-air circulation oven at 65°C for 48h when a constant weight was reached.

Germination test

All fruits from the marked branches were collected from all the selected parent trees, which presented a maximum dry mass accumulation at 40 DAA. After processing, the diaspores were stored under refrigeration

(5°C). The test was conducted in a germination chamber using 50 intact diaspores sown in transparent boxes/gerbox (11.0 × 11.0 × 3.5 cm), with four replications per treatment. The substrate consisted of blotting paper moistened with water equivalent to 2.5 times the dry substrate mass (Brasil, 2009). The diaspores were disinfected using 1% sodium hypochlorite solution (v/v) for 3 minutes.

Germination tests were conducted at temperatures of 20, 25, 30, and 20-30°C, with the presence of light. The treatment using alternating temperatures was carried out with the lowest temperature for 16h at night and the highest temperature for 8h during the day. Lighting was maintained during the high-temperature period. Germination was evaluated every 24h after the observation of the first normal seedling until germination became constant, being defined as the first and last count of the test. In addition to the germination test, the first count and germination speed index (GSI) tests (Maguire, 1962) were also performed.

Data analysis

The experimental design was completely randomized with four replications. The data were subjected to the Bartlett test to verify the homogeneity of variances. An analysis of variance was performed and, when significant, the separation of means was tested by Tukey's test at a 5% probability ($p < 0.05$) using the statistical software ASSISTAT® (Silva & Azevedo, 2016). The data on the maturation process were analyzed by the regression model with the best fit for the obtained curve.

Asexual propagation (stem cuttings)

Plant material collection site

The plant material for stem cutting production was collected in April 2018 from a natural population in southern Brazil (25°30′635″ S and 49°02′58″ W, with an altitude of 891 meters) larger than the population used for seed collection. The regional climate is also classified as Cfb, according to Alvares et al. (2013). Apical branches with leaves were collected from plants at a height of 1–2 meters from the ground from 25 female and 25 male individuals in the field during the morning (7:00-8:00 am). The branches were transported to the greenhouse in moist black polyethylene bags.

Preparation and evaluation of stem cuttings

Initially, the branches of female and male plants were disinfected separately using 0.5% sodium hypochlorite (v/v) for 15 minutes, followed by washing in water for 5 minutes. Then, apical stem cuttings measuring 8 cm long and with an average diameter of 3.0 mm and 4–6 leaves were prepared for each plant sex. The apical stem cuttings were immersed in the following concentrations of indole butyric acid (IBA) (50% aqueous ethanolic solutions (v/v): 0, 1,500, 3,000, 4,500, and 6,000 mg L^{-1} for 10 seconds. The test solution (0 mg L^{-1}) was prepared with distilled water. Subsequently, each stem cutting was placed in 120-cm^3 polypropylene tubes with the substrate MecPlant®, composed of pine bark, vermiculite, macro-and micronutrients, moisture content from 54 to 58%, and density of 375 g L^{-1}. The stem cuttings were maintained in a greenhouse set at 25 ± 2°C and 95% relative humidity under intermittent fogging for 5 seconds every 30 minutes.

After 120 days, the steam cuttings were removed from the substrates, washed in water, and evaluated for mortality (dead/necrosed cuttings), survival (live/tender cuttings with no root induction nor calluses formation), rooting, sprouting, leaf retention, callogenesis, and number and average length of roots (cm).

Data analysis

The experimental design was completely randomized in a 2×5 factorial arrangement (two sexes and five IBA concentrations), with four replicates and 16 tubes as experimental units.

Normal distribution was assumed for the average length of roots, with the data transformed with the function log (x + 0.01) before the analysis, as the graphical analysis of residuals indicated the deviations of the assumptions for analysis of variance. Then, an analysis of variance was applied to test the effect of each experimental term (main effects and interaction).

Generalized linear models with a quasi-binomial specification, which estimate a dispersion parameter, making the model more flexible to accommodate deviations from the equidispersion assumption of the overdispersion of the binomial model, were applied to the variables mortality, survival, rooting, sprouting, leaf retention, and callogenesis since they are binary variables (presence and absence of the characteristic). Generalized linear models were also applied to the variable number of roots, as it is a counting variable, but with the quasi-Poisson specification, which is more flexible because it also has a dispersion parameter. The deviation analysis for all the variables was performed using the F statistics to test the terms of the model. A regression analysis was used to study the effect of IBA doses. All analyses were performed using the statistical software R (R Core Team, 2019).

Results and discussion

Sexual propagation

The maturation process of *B. dracunculifolia* fruits, from the appearance of the first flower buds to the complete seed formation and physiological detachment from the mother plant, reached 61 days (Figure 1).

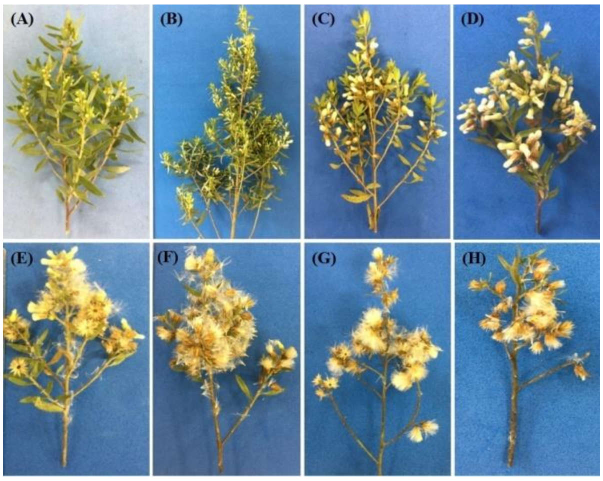

Figure 1. Characteristics of branches and diaspores of *Baccharis dracunculifolia* during physiological maturity: (A) flower buds, (B) anthesis, and (C) 19, (D) 26, (E) 29, (F) 33, (G) 36, and (H) 40 days after anthesis. Source: Maíra Maciel Tomazzoli.

The physiological maturity is reached when the diaspores have their maximum dry mass accumulation, that is, when there is no more significant transfer of reserves from the mother plant to the embryos (Bewley, Bradford, Hilhorst, & Nonogaki, 2013). The diaspores accumulated 53.85% of dry mass at 19 days after anthesis (DAA) (50% of inflorescences had open flowers), reaching 93.46% at 36 DAA and remaining virtually constant until 40 DAA (94.83%). Moreover, no diaspores were identified to be collected after five days. The increased dry mass had a significant effect of quadratic order, and the maximum point of dry mass accumulation of *B. dracunculifolia* diaspores occurred at 40 DAA (Figures 2 and 3).

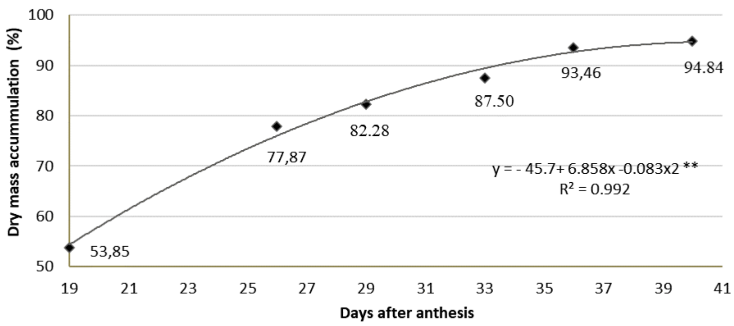

Figure 2. Dry mass accumulation of diaspores of *Baccharis dracunculifolia* throughout the maturation process. (**) Significance of P ≤ 0.01, according to the F-test.

Similar results have been found for other species of the family Asteraceae, in which the point of physiological maturity of seeds was achieved from 32 to 49 DDA (Grzybowski, Silva, Vieira, & Panobianco, 2016; Francisco, Pereira, Carvalho, & Negrelle, 2019). The initial seed dry mass accumulation is usually slow, followed by a rapid and constant accumulation until the maximum accumulation is reached and maintained. The final dry mass accumulation is mainly characterized by the interruption of the vascular connection between the seed and the mother plant, followed by the subsequent decrease in the water content of the seed and its acclimatization with the environment (Hay & Probert, 2013). However, the high dry mass index (maintenance period) is influenced by the environment, but unfavorable relative humidity and temperature conditions may contribute to accelerate the respiratory process when the seeds start the deterioration process by oxidizing the reserve substances (Bewley et al., 2013).

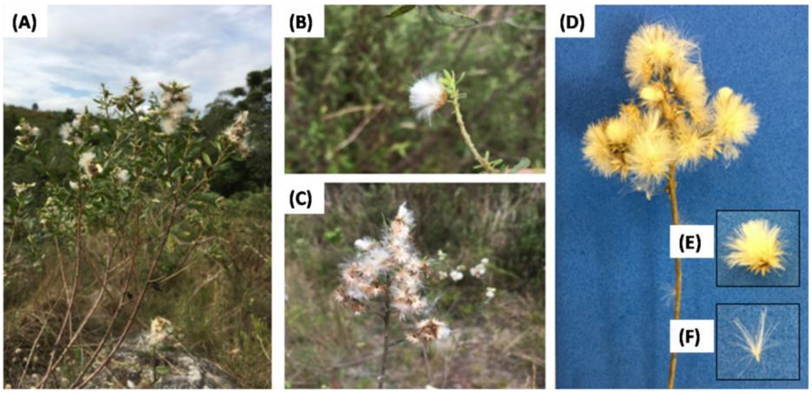

Figure 3. Branches of *Baccharis dracunculifolia* with diaspores at the optimal point of harvest (A, B, and C) and details of diaspores at the point of physiological maturity (D, E, and F). Source: Maíra Maciel Tomazzoli.

The anemochory dispersal of diaspores of *B. dracunculifolia* (Campos & Martins, 2016) may have been decisive for its rapid dispersion after reaching physiological maturity. No more diaspores were identified for the harvest after five days, which prevented the identification of decreases in the dry mass accumulation of diaspores. Therefore, changes in fruit and seed color may be a strong indication of seed maturity before dispersal (Hay & Probert, 2013).

The germination test could be studied more accurately after identifying the physiological maturity point of diaspores of *B. dracunculifolia* (Table 1). Diaspores submitted to the most extreme constant temperatures in this test (20 and 30°C) had the lowest germination, not fully reproducing the temperature behavior common to the collection region, which may have influenced the result found in this study.

Table 1. Means and standard error of the germination (%) and germination speed index (GSI) of *Baccharis dracunculifolia* diaspores under different incubation temperatures and using blotter paper as the substrate.

Temperature	Germination (%)	GSI
20°C	24 ± 2 bc	1.67 ±0.21 bc
25°C	43 ± 5 ab	3.40 ± 0.52 ab
30°C	20 ± 7 c	1.51 ± 0.53 c
20-30°C	54 ± 4 a	3.89 ± 0.29 a
C. V. (%)	28.06	29.71

Means followed by the same letter in columns do not differ by Tukey test at 5% of probability.

Santos, Figueira, and Belini (2016) tested the germination of *B. dracunculifolia* seeds submitted to 15 and 20°C and observed that 20°C with light supply provided a 50% germination rate, but higher temperatures were not tested. This result differs from that found in the present study, in which a 24% germination rate was observed at the same temperature (Table 1). On the other hand, Gomes and Fernandes (2002) tested four constant temperatures (15, 20, 25, and 30°C) using the same species and reported about 80% germination at 15 and 20°C with light supply and 15°C without light supply. Moreover, these two studies were conducted with *B. dracunculifolia* seeds collected from natural populations located in different regions, that is, the States of São Paulo (Santos et al., 2016) and Minas Gerais (Gomes & Fernandes, 2002), both in the Southeast of Brazil, thus subjected to different weather conditions and possibly different genetic material. Furthermore, many wild species have different flowering times both within the population and within individuals, which may provide a significant portion of immature seeds (Hay & Probert, 2013) and, consequently, different germination rates. The germination of this species responds positively to light and temperatures of 20-28°C, with thermo-inhibition at 35°C (Manfreda et al., 2020).

Grzybowski et al. (2016) conducted a study with seeds of *Vernonanthura discolor*, a native plant from Brazil, and reported their germination at 20 or 25°C with light supply or 25°C in the dark. According to the authors, this result was related to the pioneer characteristics of the species, whose seeds may or may not be shaded by other plants. Similarly, *B. dracunculifolia* is also considered a pioneer species, being one of the first plants to establish in the forest canopy (Barroso & Bueno, 2002), and previous studies have indicated that its seeds can germinate with or without light supply, depending on the temperature (Gomes & Fernandes, 2002; Santos et al., 2016). However, light supply is recommended in routine analysis of seeds to promote the development of essential plant structures, reduce microbial attack, and facilitate the evaluation (Brasil, 2009). Therefore, the germination of *B. dracunculifolia* was tested with a light supply only.

The constant temperatures of 25 and 30°C showed the first count at 4 days after sowing, while the temperature of 20°C and the alternating temperature of 20-30°C provided germination after 6 days (Figure 4). Vigor tests, such as first germination count and germination speed, are applied to demonstrate that physiologically aged or more deteriorated individuals have slow germination, which corresponds to the period ranging from the onset of water absorption to the emergence of the primary root. It is important because uniformity and germination speed are two factors that directly affect seedling establishment in the field (Rajjou et al., 2012). In this sense, the diaspores germinated at temperatures of 25 and 20-30°C in the present study showed the highest rates of germination speed index (Table 1), with a higher vigor than that observed at constant temperatures of 20 and 30°C. Germination speed is an important vigor attribute and an indicator of physiological quality, as delayed root emergence may be one of the first signs of deterioration (Matthews, Noli, Demir, Khajeh-Hosseini, & Wagner, 2012).

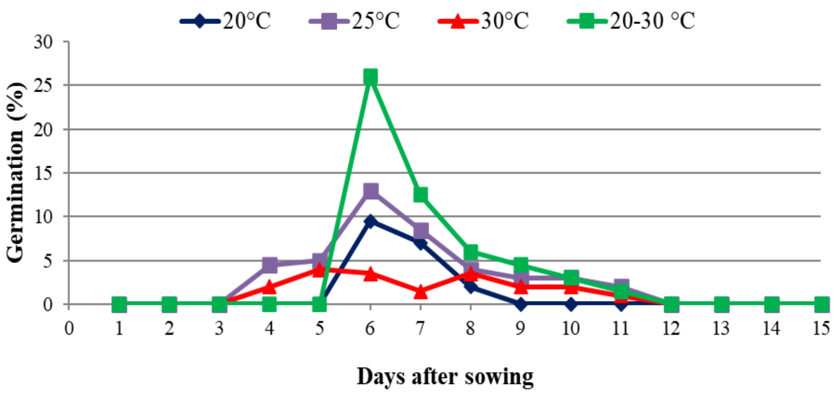

Figure 4. Percentage of germination (normal seedlings) of *Baccharis dracunculifolia* diaspores per day at 20, 25, 30, and 20–30°C.

Asexual propagation (stem cuttings)

No significant effects of sex and IBA were identified for the studied variables, but a significant dose-dependent effect for rooting, calluses, number of roots, and average length of roots was observed in the stem cuttings of *B. dracunculifolia* treated with different IBA concentrations, highlighting the effect of this auxin on root emission (Table 2). Moreover, the variables of mortality were quite high for this species although not showing significant statistical differences between treatments, ranging from 62.5 to 100% for male stem cuttings and 50 to 100% for female stem cuttings.

Table 2. Summary of the deviation analysis of rooting, sprouting, leave retention, calluses, mortality, survival, number of roots, and average length of roots of stem cuttings of *Baccharis dracunculifolia*.

Variation source	DF	Rooting		Sprouting		Leave retention		Calluses	
		Deviance	Pr(>F)	Deviance	Pr(>F)	Deviance	Pr(>F)	Deviance	Pr(>F)
Gender (F1)	1	45.351	0.424	65.857	0.586	74.783	0.340	61.339	0.384
IBA (F2)	4	29.101	0.002 **	56.741	0.161	68.824	0.496	40.857	0.012*
F1 x F2	4	24.017	0.176	45.908	0.105	60.191	0.309	39.401	0.892

Variation source	DF	Mortality		Survival		Number of roots		Average lenght of roots	
		Deviance	Pr(>F)	Deviance	Pr(>F)	Deviance	Pr(>F)	SS	Pr(>F)
Gender (F1)	1	91.747	0.760	79.863	0.300	203.906	0.036	19.914	0.900
IBA (F2)	4	87.000	0.722	74.774	0.600	105.256	0.002**	9.810	0.047*
F1 x F2	4	79.000	0.507	65.653	0.312	73.572	0.147	6.856	0.260

(**)Significant at 1% probability, according to the F-test; (*) significant at 5% probability, according to the F-test; DF = degree of freedom; SS = sum of squares; F1 = Factor 1; F2 = Factor 2. Pr(>F) = probability of observing a value for the F statistics higher than or equal to the calculated F statistics.

No other reports were found in the literature on the vegetative propagation of *B. dracunculifolia*, but a study with species of the same genus was developed by Bona et al. (2005), who evaluated the effect of different branch parts and substrates on stem cuttings of *Baccharis trimera*, *Baccharis articulata*, and *Baccharis stenocephala*, commonly known as carqueja. The authors observed that the apical and median parts of the branches are indicated for the propagation of *B. articulata* and *B. stenocephala*, whereas any part of the branch could be used to propagate *B. trimera*. Regarding the use of the substrate, sand was not recommended for the species.

Although vegetative propagation is usually more costly than seed reproduction (Hartmann et al., 2011), this reproduction method is interesting for *B. dracunculifolia* because it assists in the selection of superior clones regarding the production of secondary metabolites, justifying the higher propagation costs.

The lowest rooting of stem cuttings occurred due to the absence of IBA (Figure 5). An increase in the percentage of rooting is related to an increase in IBA doses. In fact, the percentage of rooting of stem cuttings of *B. dracunculifolia* reached 0.47% without the application of this auxin, while the application of 6,000 mg L^{-1} increased this percentage to 7.61%. The decrease in root formation as a function of the IBA application is shown in Figure 5.

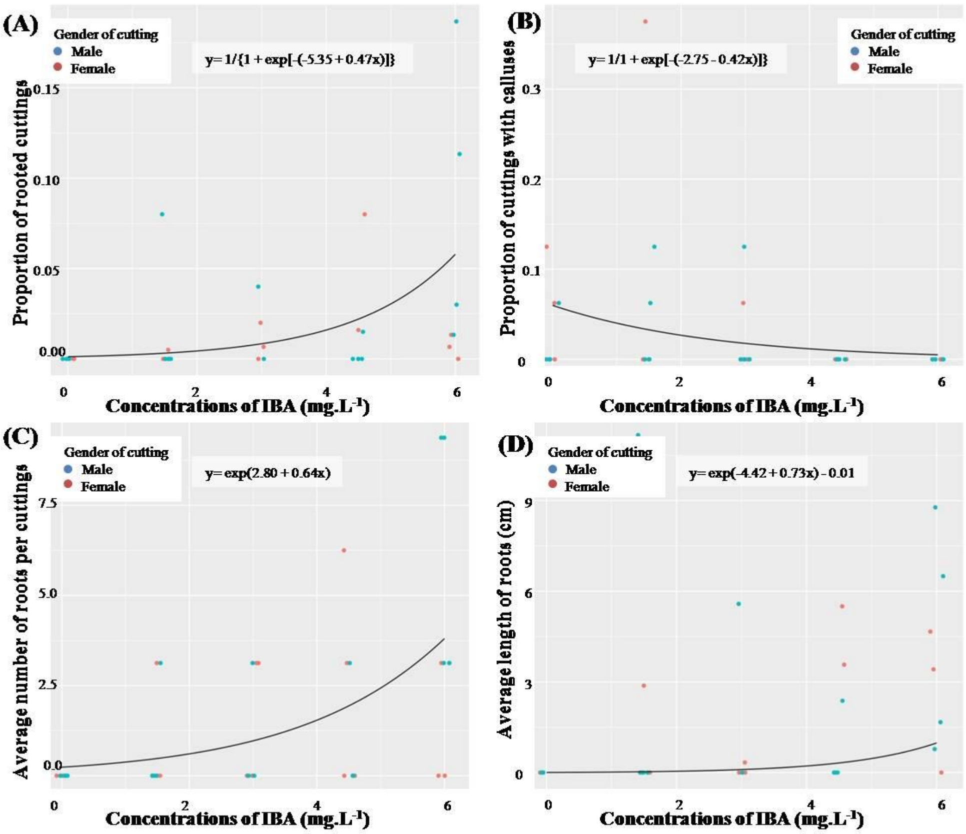

Figure 5. Proportion of rooted cuttings (A), proportion of cuttings with calluses (B), average number of roots per cutting (C), and average length of roots (cm) (D) in stem cuttings of *Baccharis dracunculifolia* treated with increasing IBA concentrations (mg L^{-1}).

The number of roots per cutting and length (cm) presented a positive exponential increase as IBA doses increased (Figure 5C-D). In addition to rooting, other factors such as the number and length of roots are also important for the formation of quality seedlings (Hartmann et al., 2011).

The percentage of formation of calluses in cuttings of *B. dracunculifolia* reduced with an increase of IBA doses. Moreover, this percentage reached 5.98% with no application of IBA and reduced to 0.509% with an increasing IBA application of up to 6,000 mg L^{-1}. The regression of the formation of calluses as a function of IBA application (negative function) is shown in Figure 5B. Calluses usually are developed in the basal region of cuttings and are defined as an irregular mass of parenchyma cells whose growth proliferates primarily from cells of the vascular cambium and also from cells of the cortex and pith (Hartmann et al., 2011).

However, variations in the rooting potential of plant species within the same genus are expected. Bona et al. (2005) reported high rooting rates for *B. trimera* and *B. stenocephala*, with values of 76.1 and 83.3%, respectively, even with no application of exogenous auxin. Similar results have been observed for species of other genera, such as *Piper* spp., in which the species *Piper crassinervium* had a high rooting rate of 75.83% (Nunes Gomes & Krinski, 2019) and *P. amalago* showed a rooting rate of only 22.92% (Nunes Gomes & Krinski, 2016). These variations may be related to several factors that act in isolated or concomitant ways, such as the amount of endogenous auxin and other rooting promoters intrinsic to each species, the type of cuttings, time of collection of plant material, and humidity, temperature, and light conditions (Zuffellato-Ribas & Rodrigues, 2001).

The root formation process is usually divided into four stages: i) dedifferentiation, i.e., the activation of cells to become competent; ii) root formation from cells of the vascular tissue, where they become meristematic by dedifferentiation; iii) meristematic cells (development of root initials) that are developed into organized root primordia; and iv) root primordia, which grow and emerge out of the stem tissue and formation of vascular tissue between the vascular tissue of the cutting and the root primordia (Hartmann et al., 2011).

Auxins such as IAA and IBA are important phytohormones that regulate the induction of adventitious roots (Steffens & Rasmussen, 2016). However, auxin is only part of the stimulus, as the root formation for many species with difficult rooting has been improved through the addition of rooting cofactors, which are endogenous substances capable of acting synergistically with IAA promoting the rooting of cuttings (Zuffellato-Ribas & Rodrigues, 2001). The complete understanding of the role of these cofactors in the induction of roots is complicated due to the apparent diversity of this group of bioactive substances. Previous studies have shown that some exogenous catechol-related polyphenols can act as rooting promoters in the same way that some monophenols can act as IAA-oxidase cofactors to reduce root induction (Zuffellato-Ribas & Rodrigues, 2001).

The rooting of cuttings of *B. dracunculifolia* presented in this study may also be associated with anatomical barriers for root emission related, for example, to the formation of a continuous sclerenchyma ring between the phloem and the cortex, which occurs in species with difficult rooting, while species that are easily rooted are characterized by the discontinuity of the sclerenchyma ring or the lower amount of its cell layers (Hartmann et al., 2011).

This is a previous study of *B. dracunculifolia* propagation through stem cuttings, but there is a need to optimize the methodology due to the high mortality and low rooting of the cuttings even at IBA concentrations considered high. Although the analysis indicates an increase in the rooting of cuttings with this auxin application, tests with increased doses would not be the most indicated, as the excessive application of auxins may cause stem blackening and cutting death (Hartmann et al., 2011).

Some factors may be involved in this low performance and help to justify the reduction of the survival and rooting of cuttings, such as the phenological stage of the mother-plant, as the phenological stage can affect the rooting capacity of species with difficult rooting, such as the transition from the vegetative to the flowering stage, given the competition between flowering and rooting (Rasmussen, Hosseini, Hajirezaei, Druege, & Geelen, 2015). In this sense, further studies on the vegetative propagation of *B. dracunculifolia* are suggested to be developed considering its seasonal behavior of the propagation by cuttings. Another alternative is the production of mini-cuttings using propagules originated from seeds, given the rejuvenation of the plant material and the high concentration of endogenous auxin, as previously described for the species *Araucaria angustifolia* (Pires, Wendling, & Brondani, 2013). In addition, the application of rejuvenation techniques, such as the propagation of epicormic buds, serial propagations, and the collection of branches from different parts of the plant can be alternatives to increase the rooting rate of cuttings (Wendelling, Treuman, & Xavier, 2014).

Conclusion

The physiological maturity of *B. dracunculifolia* diaspores occurred at 40 days after anthesis, being the best time for collection in the field. The constant temperature of 25 or 20-30°C should be used in the germination test, with the first count at four days after sowing and the last count after 11 days. In this study, *B. dracunculifolia* was a species with difficult rooting when using stem cuttings. The factor plant sex did not influence rooting, but the increment in IBA concentration increased the rooting of this species although the methodology optimization is necessary.

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