



Effect of Cutting Size on the Performance of Exogenous Auxin IBA in Vegetative Propagation of *Rubus erythroclados* Mart. ex Hook. f.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The climbing shrub *Rubus erythroclados* Mart. ex Hook. f. (*amora-verde*) has great food (fruits) and medicinal (leaves) potential. However, this species remains understudied. The objective of this study was to evaluate the interaction between cutting size and IBA concentration in the vegetative propagation of *R. erythroclados* by stem cuttings.

Study Design: The experimental design used was completely randomized in a 3x5 factorial combination. The cutting size factor was represented by three levels (10, 15 and 20 cm) and IBA concentration factor by five levels (0, 1,000, 2,000, 3,000 and 4,000 mg L⁻¹).

Results: There was an interaction between the factors in 13 of the 19 variables analyzed, being: live cuttings at 30 and 60 days, live cuttings with callus formation, sprouted cuttings at 30, 60 and 90 days, rooted cuttings, shoot number, shoot diameter, root number, root length, length of the largest root and root diameter. Size increase of the *R. erythroclados* cuttings caused a decrease in the phytotoxic effect of IBA in the development of the aerial part. The effect of IBA in response to rooting induction, in the larger size of *R. erythroclados* cuttings, required an increase in IBA concentration compared to the cuttings of shorter size.

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Conclusion: Cuttings of 10 and 15 cm treated with 1,000 mg L⁻¹ of IBA showed greater rooting (20.0%) compared to the other treatments, being, therefore, indicated for the vegetative propagation of *R. erythroclados* by stem cuttings.

Keywords: Endemic species; fruticulture; medicinal species; non-domesticated species; small fruit.

1. INTRODUCTION

The *Rubus* genus, belonging to the Rosaceae family, is one of the most diverse in the plant kingdom, presenting global distribution [1]. There are seven species of native *Rubus* in Brazil, but the species *R. erythroclados* stands out [2]. Popularly known as “amora-verde” or “amora-branca”, *R. erythroclados* is a climbing shrub with young red branches and numerous thorns. The lower leaves have five leaflets, while the upper leaves have three leaflets. The flowers are small and arranged in terminal panicles. The fruit is botanically classified as an aggregate of drupelets and, when ripe, presents a light green color [2].

Currently, this species is described as an unconventional food plant due to its green fruits with high sugar levels and pleasant taste [3, 4]. *R. erythroclados* has the potential to be among the main small fruits grown in Brazil together with blackberry and raspberry because of the quality of its fruits [5]. In addition, the presence of the flavonoids quercetin and kaempferol in the leaves provides the species with great medicinal purposes [6], usually consumed in the form of tea leaves. However, the literature lacks studies involving *R. erythroclados*, which is difficult for the creation of domestication programs and commercial cultivation of the species.

Vegetative propagation by stems and root cuttings are the main forms of propagation used in *Rubus* species because it is possible to obtain large amounts of genetically identical seedlings in a short period [7]. Cutting size and the application of indolebutyric acid (IBA) significantly influence the rooting process and consequently the obtaining of seedlings using the cutting technique [8]. However, there are no studies in the literature involving *Rubus* species evaluating the interaction between cutting size and IBA concentrations.

Despite the great potential of *R. erythroclados*, there is only one study in the literature involving the propagation of this species [9]. The rooting percentage of 60.0% was found in stem cuttings of *R. erythroclados* treated with 1,000 mg L⁻¹, in

an experiment performed in the summer. However, the vegetative propagation of *Rubus* species by stem cuttings should be performed in the winter period, during dormant pruning [10].

In this context, the objective of this study was to evaluate the interaction between cutting size and IBA concentration in the vegetative propagation of *R. erythroclados* by stem cuttings during the winter period.

2. MATERIALS AND METHODS

2.1 Description of the Study Site

The experiment was carried out from July to October 2020 in a greenhouse in the “Setor de Fruticultura do Instituto Federal do Rio Grande do Sul (IFRS) - Campus Sertão” (28° 02' 42" S, 52° 16' 17" W and 737 m.a.s.l.).

Semi-hardwood stem cuttings were used, taken from the middle part of the branches of *R. erythroclados* plants with one year of age, from a home orchard located in the municipality of Getúlio Vargas, South Brazil (27° 52' 37" S, 52° 13' 35" W and 665 m.a.s.l.). The climate of the region, based on the Köppen classification, is Cfa (humid subtropical) with an average temperature of 17.7°C and annual rainfall of 1,803.1 mm [11]. The local soil is classified as dystrophic Red Latosol (Oxisol) [12].

2.2 Experimental Design

The experimental design was completely randomized, with four replications and 10 cuttings per experimental unit, in a 3x5 factorial combination, with the cutting size factor represented by three levels (10, 15 and 20 cm) and the IBA concentration factor, for five levels (0, 1,000, 2,000, 3,000 and 4,000 mg L⁻¹), totaling 600 cuttings.

2.3 Methodology

The cuttings were standardized by size (10, 15 and 20 cm) and diameter (around 8 mm), without leaves, with horizontal cut at the base and bevel

at the upper end. After, the cuttings were immersed in IBA for 10 seconds in concentrations of 1,000, 2,000, 3,000 and 4,000 mg L⁻¹. Distilled water was used for treatment control.

The cuttings were buried 2/3 of their length in a vertical position. The cuttings were planted in plastic boxes filled with fine granule vermiculite, placed in a greenhouse, with daily misting irrigation every 2 hours, for 1 minute, from 6:00 am to 6:00 pm.

2.4 Analyzed Variables

The following variables were evaluated: live cuttings at 30 and 60 days (%), sprouted cuttings at 30 and 60 (%), live cuttings without callus formation (%), live cuttings with callus formation (%), sprouted cuttings (%), rooted cuttings (%), shoot number, leaf number, shoot length (mm), shoot diameter (mm), shoot dry matter (g), root number, root length (mm), length of the largest root (mm), total root length (mm), root diameter (mm) and root dry matter (g) at 90 days.

2.5 Data Analysis

The data were subjected to a normality test to check for possible transformation needs, which were not detected. Then, they were subjected to

analysis of variance and the means compared by the Tukey test ($\alpha = 0.05$), using the software Sisvar 5.6.

3. RESULTS AND DISCUSSION

The live and sprouted cuttings percentages, both at 30 and 60 days after the implementation of the experiment, were higher in 20 cm cuttings than 10 cm cuttings (Table 1). Therefore, there was a tendency to increase the live and sprouted cuttings percentages as cutting size rose. The high nutrient content in larger cuttings [8] was possibly the cause of greater live and sprouted cuttings percentages than shorter cuttings that quickly depleted their reserves.

The live and sprouted cuttings percentages, both at 30 and 60 days after the implementation of the experiment, were greater in cuttings with or without application of low concentrations of IBA compared to cuttings with application of high concentrations of IBA (Table 1). As IBA concentration increased, the live and sprouted cuttings percentages decreased because of the phytotoxic effect of exogenous auxin IBA [13]. It demonstrates that the *R. erythroclados* species is quite sensitive to this hormone compared with the cuttings survival and sprouting, not tolerating even low IBA concentrations. Therefore, exogenous auxin IBA has an herbicide effect on the vegetative development of *R. erythroclados*.

Table 1. Percentages of live cuttings at 30 and 60 days and sprouted cuttings at 30 and 60 days of *Rubus erythroclados* after the implantation of the experiment

Factors	Variables			
	Live cuttings at 30 days (%)	Live cuttings at 60 days (%)	Sprouted cuttings at 30 days (%)	Sprouted cuttings at 60 days (%)
Cutting size (cm) (A)				
10	96.0 b ¹	59.5 b	50.0 b	45.0 c
15	99.0 a	82.5 a	70.0 a	70.5 b
20	96.5 ab	86.0 a	72.5 b	80.5 a
IBA concentration (mg L⁻¹) (B)				
0	100.0 a	87.5 a	70.0 a	76.6 a
1,000	95.0 b	83.5 a	59.1 c	73.3 a
2,000	97.5 ab	73.3 b	65.8 ab	64.1 b
3,000	98.3 ab	74.1 b	63.3 bc	57.5 c
4,000	95.0 b	62.5 c	63.3 bc	55.0 c
F test				
A	51.6 *	4145.0 **	2901.6 **	6701.6 **
B	56.6 **	1101.6 **	189.1 **	1085.8 **
AxB	57.9 **	267.9 **	301.6 **	418.3 **
CV (%)	3.8	6.2	7.6	7.2

¹Means followed by different letters in the same column, differ to 5% of probability of error by Tukey test; * significant at 5% and ** significant at 1% probability of error; ns - not significant

There was a significant interaction between cutting size and IBA concentration for the live and sprouted cuttings variables at 30 and 60 days (Table 1).

In general, as cutting size of *R. erythroclados* increased, the phytotoxic effect caused by high concentrations of IBA was reduced in the live cutting percentages at 30 (Fig. 1A) and 60 days (Fig. 1B), as well as in the sprouted cutting at 30 (Fig. 1C) and 60 days (Fig. 1D) after the implementation of the experiment. Longer cuttings attenuate the phytotoxic effect of exogenous auxin IBA on the survival and sprouting of cuttings. Thus, as a cutting increase, there is a greater dilution of this hormone in the plant [13].

Size increase of *R. erythroclados* cuttings resulted in the growth of the live cuttings without callus formation percentage. In contrast, the

increase in IBA concentration reduced the live cuttings without callus formation percentage after 90 days of the experiment implantation (Table 2). There was no significant interaction between cutting size and IBA concentration for the live cuttings without callus formation variable.

Cutting size did not influence the live cuttings with callus formation percentage after 90 days of the experiment implantation (Table 2). The application of IBA had a negative influence on the formation of callus. In cuttings without application, greater callus formation was observed than the cuttings subjected to higher concentrations of IBA.

Although callus formation and root emission are distinct processes, there is a tendency for root emission to occur through the differentiation of callus parenchymal cells [8]. However, when in excess, the callus can impair root emission.

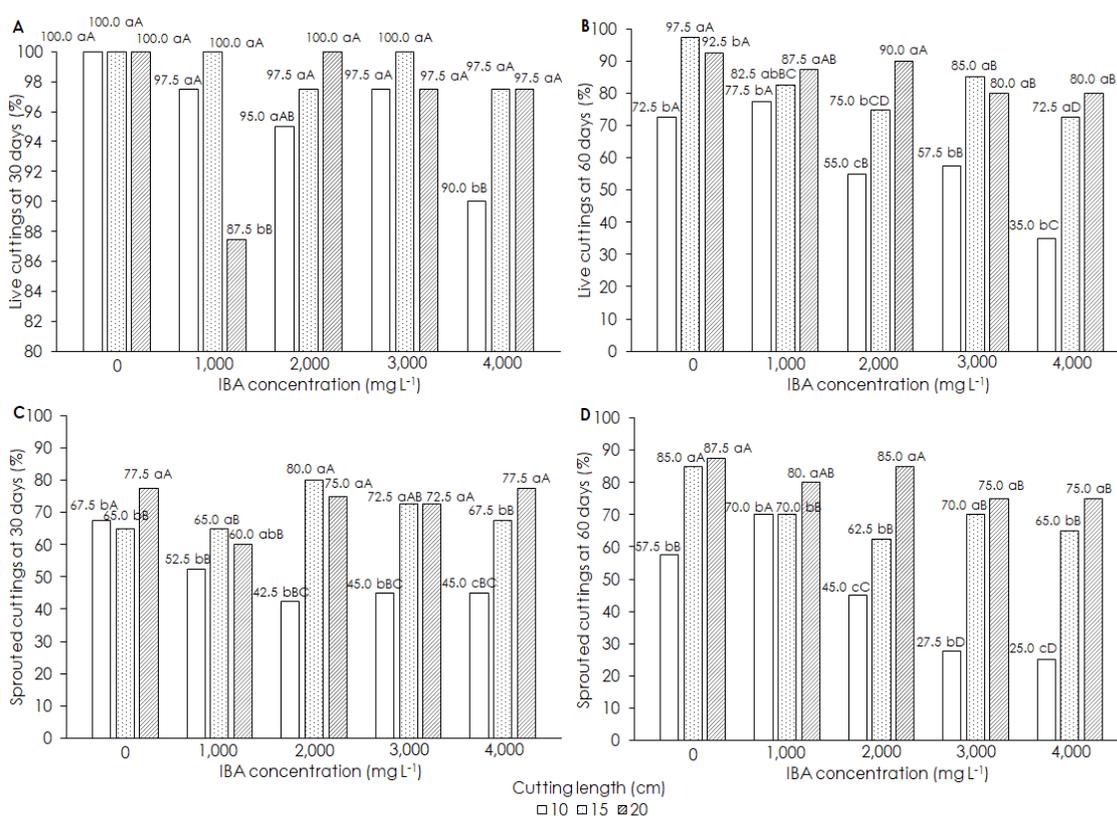


Fig. 1. Live cuttings (%) at 30 (A) and 60 (B) days and sprouted cuttings (%) at 30 (C) and 60 (D) days in the cutting size x IBA concentration interaction

* Lowercase letters compare the cutting sizes within each IBA concentration and uppercase letters compare the IBA concentrations within each cutting sizes, where averages with the same letters do not differ from each other significantly by the Tukey test ($p < 0.05$)

Table 2. Percentages of live cuttings without and with callus formation, sprouted cuttings and rooted cuttings of *Rubus erythroclados* 90 days after the implantation of the experiment

Factors	Variables			
	Live cuttings without callus formation (%)	Live cuttings with callus formation (%)	Sprouted cuttings (%)	Rooted cuttings (%)
Cutting size (cm) (A)				
10	10.0 b ¹	5.0 a	16.0 b	7.0 b
15	28.0 a	6.5 a	44.5 a	11.5 a
20	30.0 a	6.0 a	38.5 b	6.5 b
IBA concentration (mg L⁻¹) (B)				
0	35.8 a	14.1 a	49.1 a	6.6 b
1,000	21.6 b	5.0 b	35.8 b	15.0 a
2,000	20.8 bc	4.1 b	28.3 b	5.8 b
3,000	19.1 bc	3.3 b	25.8 b	7.5 b
4,000	15.8 c	2.5 b	25.8 b	6.6 b
F test				
A	2426.6 **	11.6 ns	4515.0 **	151.6 **
B	710.0 **	270.8 **	1181.6 **	170.8 **
AxB	35.0 ns	47.0 *	187.9 *	162.0 **
CV (%)	20.8	79.8	27.1	48.9

¹Means followed by different letters in the same column, differ to 5% of probability of error by Tukey test; * significant at 5% and ** significant at 1% probability of error; ns - not significant

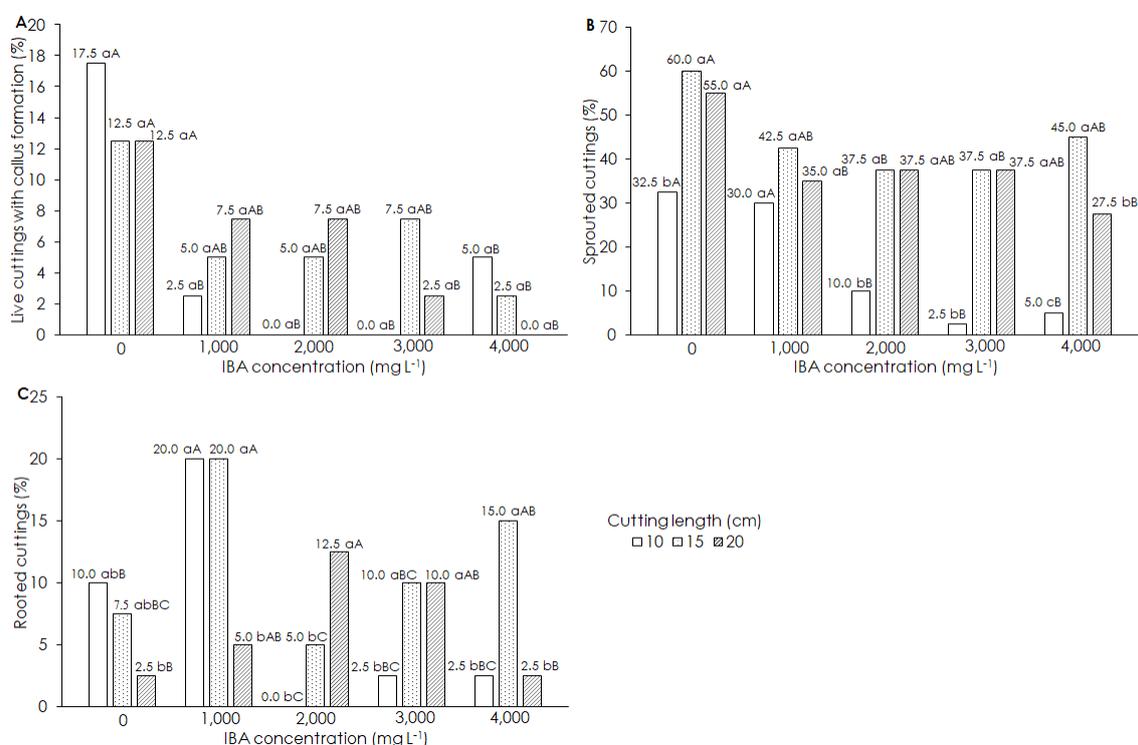


Fig. 2. Live cuttings with callus formation (%) (A), sprouted cuttings (%) (B) and rooted cuttings (%) (C) in the cutting size x IBA concentration interaction

* Lowercase letters compare the cutting sizes within each IBA concentration and uppercase letters compare the IBA concentrations within each cutting sizes, where averages with the same letters do not differ from each other significantly by the Tukey test ($p < 0.05$)

There was a significant interaction between cutting size and IBA concentration for the live cuttings with the callus formation variable (Table 2). We observed that the highest values of live

cuttings with callus formation were concentrated in the cuttings with no application of IBA (Fig. 2A). In addition, the increase in cutting size was able to reduce, although in a not so pronounced way, the negative effect of IBA on callus formation.

The sprouted cuttings percentage at 90 days after implantation of the experiment, also observed at 30 and 60 days, showed higher values in the longest cuttings and lower values in the cuttings as IBA concentration increased (Table 2). At 90 days, the occurrence of significant interaction between size cutting and IBA concentration for the variable sprouted cuttings reinforces, once again, the attenuating effect that size cutting cut has on the phytotoxicity of the IBA (Fig. 2B).

Regarding rooted cuttings, cuttings of 15 cm showed a higher percentage compared to those of 10 and 20 cm (Table 2; Fig. 3). Although the emission of adventitious roots in cuttings of greater size is favored by large amounts of carbohydrates present in these cuttings [8], the rooting of cuttings of 20 cm in length of *R. erythroclados* was possibly impaired due

to more significant dehydration than the shorter ones.

There are no studies in the literature involving *Rubus* species evaluating the effect of different sizes of cutting on rooting. Considering other botanical genera, the results are quite diverse. In *Baccharis trimera* (Less.) DC. [14], *Lippia sidoides* Cham. [15], *Ficus carica* L. [16] and *Tropaeolum pentaphyllum* Lam. [17] greater rooting was observed as cutting length increased, whereas in *Rhododendron simsii* Planch. [18] and *Lagerstroemia indica* L. [19] shorter cuttings showed greater rooting. In *Hyptis suaveolens* (L.) Poit. [20], cutting length did not influence the rooting of cuttings.

The application of 1,000 mg L⁻¹ of IBA caused a higher percentage of rooting than the non-application of IBA and the application of concentrations greater than 1,000 mg L⁻¹ of IBA (Table 2). The species *R. erythroclados* possibly contains insufficient endogenous concentrations of auxin to promote a natural rooting of cuttings, requiring the exogenous application of IBA [9]. However, when exceeding 1,000 mg L⁻¹ IBA, there is a negative effect on rooting.



Fig. 3. Rooting of *Rubus erythroclados* cuttings with 10 (A), 15 (B) and 20 (C) cm in length treated with 0, 1,000, 2,000, 3,000 and 4,000 mg L⁻¹ of IBA 90 days after the implementation of the experiment

Each plant species has an ideal auxin value that makes the rooting of cuttings possible; thus, the application of exogenous auxin should complement the levels of endogenous auxin when necessary [21].

In the *Rubus* genus, such variation in IBA response to rooting is verified. In experiments with blackberry (*Rubus* sp.) [22], hill raspberry (*R. niveus* Thunb.) [23], blackberry (*R. fruticosus* L.) [7], blackberry (*Rubus* sp. cv. Brazos) [24], boysenberry (interspecific hybrid between *R. loganobaccus* L.H. Bailey and *R. baileyanus* Britton) [25], higher rooting percentages were found without the application of IBA. In blackberry (*Rubus* sp.), higher rooting percentages were obtained with applications of 2,000 [26] and 1,000 mg L⁻¹ [10] of IBA.

There was a significant interaction between size cutting and IBA concentration for the rooted cuttings variable (Table 2). The application of 1,000 mg L⁻¹ IBA in the 10 and 15 cm cuttings promoted greater rooting (20.0%) than the other treatments (Fig. 2C). The 10 cm cuttings treated with 2,000 mg L⁻¹ IBA were the only treatment that did not show rooting (Fig. 3).

There is a tendency towards the need to increase IBA concentration to promote rooting as cutting length increased (Fig. 2C) due to the more significant dilution of exogenous auxin IBA in cuttings of greater size [8]. Therefore, to obtain the desired rhizogenic effect, it is necessary to increase IBA concentration.

The rooting percentages of *R. erythroclados* cuttings found in this study are low when compared to other *Rubus* species in which rates obtained were more than 50% [7, 10, 22, 23, 24, 25, 26, 27]. It is important to emphasize that *R. erythroclados* is a native species that has not yet been domesticated, so there is a need to improve the cutting technique or test other propagation forms.

The results found in this study regarding rooting of cuttings are also lower than that of Balestrin et al. [9], in which a rooting of 60.0% in cuttings of *R. erythroclados* of 10 cm treated with 1.000 mg L⁻¹ of IBA. Such discrepancy may be due to the time of year when cuttings were collected from the mother plants. These authors collected cuttings in late spring, while in the present study, cuttings were collected in winter when the gems are dormant.

Although the study by Balestrin et al. [9] showed a greater rooting when cuttings are collected in late spring, it is important to note that the *R. erythroclados* plants are in the reproductive phase during this season. Therefore, the propagation by stem cuttings can significantly compromise fruit production, since it removes the branches from the plant that are potential fruit producers.

Therefore, the collection of cuttings of *R. erythroclados* in winter, performed in this study, has as its primary objective not to harm fruit production. Moreover, the collection of cuttings in winter (during pruning) is a way to take advantage of the branches removed from the plants, since pruning is indispensable for the temperate of fruit species [5], such as *R. erythroclados*.

One possibility to increase the rooting of *R. erythroclados*, when the propagation by stem cuttings is performed in winter, is to increase the period of cuttings remaining in the substrate. At 90 days after the implementation of the experiment, we observed the presence of live cuttings (without or with callus formation), which may indicate that the rooting of cuttings in *R. erythroclados* could occur beyond this period. However, it is important to emphasize that a longer permanence of the cuttings in the substrate is not a guarantee of rooting, given that the cuttings tend to senescence over time when the reserves are depleted.

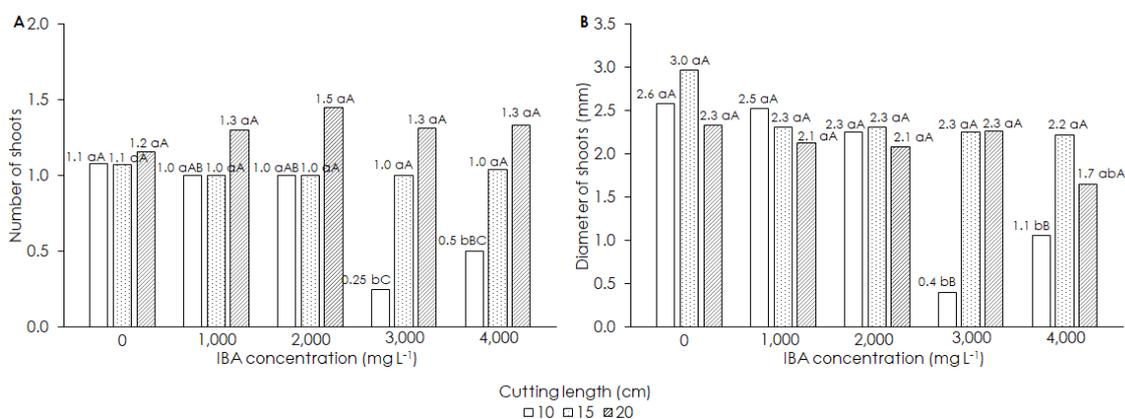
All the variables analyzed in relation to the aerial part of *R. erythroclados* cuttings (shoot number, leaf number, shoot length, shoot diameter and shoot dry matter), at 90 days after the experiment implementation, showed higher values in the cuttings of greater size (Table 3). It shows that the cuttings of greater size, besides having greater sprouting (Table 1), demonstrated more significant development of the aerial part.

Regarding the application of IBA, except for shoot dry matter that did not differ between treatments, all the variables evaluated in relation to the aerial part of *R. erythroclados* cuttings showed a tendency to decrease the values as IBA concentration increased (Table 3), reinforcing once again, the herbicide effect of IBA in the aerial part of *R. erythroclados* cuttings.

Table 3. Number of shoots, number of leaves, length of shoots (mm), diameter of shoots (mm), dry matter of shoots (g) of *Rubus erythroclados* cuttings 90 days after the implantation of the experiment

Factors	Variables				
	Number of shoots	Number of leaves	Length of shoots (mm)	Diameter of shoots (mm)	Dry matter of shoots (g)
Cutting size (cm) (A)					
10	0.7 c ¹	2.6 b	18.7 b	1.7 b	0.1 c
15	1.0 b	3.9 a	41.8 a	2.4 a	0.2 b
20	1.3 a	4.8 a	52.7 a	2.0 ab	0.3 a
IBA concentration (mg L⁻¹) (B)					
0	1.1 a	4.4 a	44.1 a	2.6 a	0.2 a
1,000	1.1 a	4.3 a	38.8 a	2.3 a	0.2 a
2,000	1.1 a	3.9 a	38.0 a	2.2 ab	0.2 a
3,000	0.8 a	3.0 a	34.9 a	1.6 b	0.2 a
4,000	0.9 a	3.2 a	33.5 a	1.6 b	0.2 a
F test					
A	1.5 **	25.9 **	6053.6 **	2.1 **	0.3 **
B	0.1 ns	4.8 ns	200.0 ns	2.2 **	0.005 ns
AxB	0.2 *	2.0 ns	163.0 ns	1.1 **	0.007 ns
CV (%)	27.1	33.8	39.7	24.0	37.9

¹Means followed by different letters in the same column, differ to 5% of probability of error by Tukey test; * significant at 5% and ** significant at 1% probability of error; ns - not significant

**Fig. 4. Number (A) and diameter (B) of shoots (mm) in the cutting size x IBA concentration interaction**

* Lowercase letters compare the cutting sizes within each IBA concentration and uppercase letters compare the IBA concentrations within each cutting sizes, where averages with the same letters do not differ from each other significantly by the Tukey test ($p < 0.05$)

Of the aerial part variables analyzed, only shoot number and shoot diameter showed a significant interaction between cutting size and IBA concentration factors (Table 3). Both shoot number (Fig. 4A) and shoot diameter (Fig. 4B) variables demonstrated a reduction in the phytotoxic effect on the aerial part as the cutting size increased.

Regarding the root system of *R. erythroclados* cuttings, root length, length of the largest root, total root length, and root dry matter variables

were not influenced by cutting size and IBA concentration factors when analyzed separately (Table 4).

Root number was influenced only by cutting size, where a higher value was observed in the cuttings of 15 cm than the others (Table 4).

Root diameter was influenced by cutting size and the application of IBA, in which the highest values were observed in the 15 cm cuttings and the treated cuttings 1,000 mg L⁻¹ IBA (Table 4).

The root system variables that showed a significant interaction between the cutting size and IBA concentration factors were: root number, root length, length of the largest root, and root diameter (Table 4). In these variables, the

application of IBA causes different responses. In general, it is necessary an increase of IBA concentration in the cuttings of greater size to induce a positive reaction of IBA to the rooting (Fig. 5).

Table 4. Number and length of roots (mm), length of the largest root (mm), length of total roots (mm), diameter of roots (mm) and dry matter of roots (g) of *Rubus erythroclados* cuttings 90 days after the implantation of the experiment

Factors	Variables					
	Number of roots	Length of roots (mm)	Length of the largest root (mm)	Length of total roots (mm)	Diameter of roots (mm)	Dry matter of roots (g)
Cutting size (cm) (A)						
10	1.7 b ¹	14.3 a	20.2 a	57.6 a	0.5 b	0.005 a
15	4.9 a	27.5 a	41.6 a	193.3 a	0.9 a	0.031 a
20	2.8 ab	16.9 a	24.8 a	127.3 a	0.5 b	0.027 a
IBA concentration (mg L⁻¹) (B)						
0	1.4 a	15.0 a	20.0 a	30.3 a	0.7 ab	0.001 a
1,000	4.3 a	25.4 a	34.7 a	153.0 a	0.9 a	0.023 a
2,000	2.1 a	16.0 a	22.6 a	86.4 a	0.4 b	0.020 a
3,000	4.7 a	21.1 a	34.5 a	209.2 a	0.7 ab	0.042 a
4,000	3.3 a	20.2 a	32.6 a	151.5 a	0.4 b	0.021 a
F test						
A	53.2 *	980.6 ns	2540.8 ns	92126.0 ns	1.0 **	0.003 ns
B	24.1 ns	210.0 ns	595.4 ns	57053.2 ns	0.5 *	0.002 ns
AxB	33.8 *	962.4 *	2416.1 *	84309.4 ns	0.9 **	0.003 ns
CV (%)	110.9	103.3	112.5	157.8	58.7	219.1

¹Means followed by different letters in the same column, differ to 5% of probability of error by Tukey test; * significant at 5% and ** significant at 1% probability of error; ns - not significant

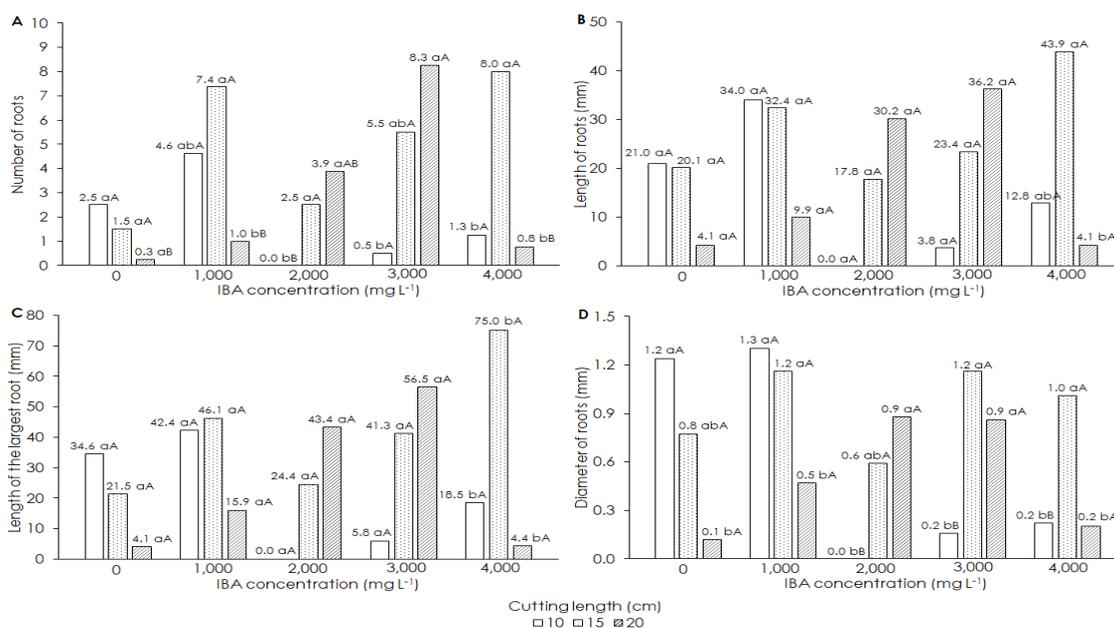


Fig. 5. Number of roots (A), length of roots (mm) (B), length of the largest root (mm) (C) and diameter of roots (mm) (D) in the cutting size x IBA concentration interaction

* Lowercase letters compare the cutting sizes within each IBA concentration and uppercase letters compare the IBA concentrations within each cutting sizes, where averages with the same letters do not differ from each other significantly by the Tukey test ($p < 0.05$)

4. CONCLUSION

Vegetative propagation in *R. erythroclados* by cuttings of 10 and 15 cm treated with 1,000 mg L⁻¹ of IBA showed greater rooting (20.0%) than the other treatments.

In general, the interaction between cutting size and IBA concentration factors, when it occurred, caused a reduction in the phytotoxic effect of high concentrations of IBA in the aerial part variables as the cutting size increased. The positive response of IBA to the induction to rooting required, in the cuttings of greater size, an increase of IBA concentration compared with the cuttings of shorter size.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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