

## SCIENTIFIC ARTICLE

## Rooting of azalea cuttings of Otto and Terra Nova cultivars treated with auxin and boron

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### Abstract

Azalea (*Rhododendron simsii* Planch.) is an ornamental plant native to China, propagated commercially by cuttings and cultivated in Brazil due to the beauty of its flowers. However, it presents rooting difficulties, which can lead to a reduction in the commercial production of these seedlings. Thus, the application of plant regulators is necessary to achieve rooting success as well as of nutrients that are involved in biochemical and physiological plant processes. This work aimed to evaluate the rooting performance of Otto and Terra Nova azalea herbaceous cuttings treated with indolebutyric acid (IBA) and boron. The experiments were conducted at a private property specialized in the production of potted azalea, located in Holambra County, São Paulo State, Brazil, and the evaluations were carried out at the Plant Seed Laboratory of the Crop Production Department, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal. The experimental design for each experiment with each cultivar was completely randomized. The treatments were arranged in a 4x4 factorial scheme (IBA concentrations x boron concentrations) with four replications and 10 cuttings per replication. The evaluation was performed six weeks after experiment beginning. Auxin and boron did not influence rooting percentage of cuttings of both cultivars. However, Otto cultivar presented a better-quality root system when cuttings were treated with 2,000 mg L<sup>-1</sup> IBA in the absence of boron.

**Keywords:** *Rhododendron simsii*, micronutrient, indolebutyric acid, rhizogenesis.

### Resumo

#### Enraizamento de estacas de azaleia, cultivares Otto e Terra Nova, tratadas com auxina e boro

A azaleia (*Rhododendron simsii* Planch.) é uma planta ornamental originária da China, propagada comercialmente por estacas e muito cultivada no Brasil devido à beleza de suas flores. No entanto, apresenta dificuldades no enraizamento, o que pode provocar uma redução na produção comercial dessas mudas. Dessa forma, é necessária a aplicação de fitorreguladores para alcançar o sucesso do enraizamento, bem como a utilização de nutrientes que estejam envolvidos nos processos bioquímicos e fisiológicos das plantas. O objetivo do trabalho foi avaliar a influência do ácido indolbutírico (AIB) e do boro no enraizamento de estacas herbáceas das cultivares de azaleia Otto e Terra Nova. Os experimentos foram realizados em uma propriedade particular especializada na produção de azaleias em vasos, localizada em Holambra/SP, e as avaliações foram realizadas no Laboratório de Sementes e Plantas Hortícolas do Departamento de Produção Vegetal, da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista (FCAV/UNESP), em Jaboticabal/SP. O delineamento experimental, para cada experimento com cada cultivar, foi o inteiramente casualizado. Os tratamentos foram arranjados em esquema fatorial 4x4 (concentrações de AIB x concentrações de boro) com quatro repetições e 10 estacas por repetição. A avaliação foi realizada seis semanas após o estaqueamento. Concluiu-se que a auxina e o boro não influenciaram na porcentagem de enraizamento das estacas para ambos as cultivares. No entanto, a cultivar Otto apresentou um sistema radicular de melhor qualidade quando as estacas foram tratadas com 2.000 mg L<sup>-1</sup> de AIB na ausência de boro.

**Palavras-chave:** *Rhododendron simsii*, micronutriente, ácido indolbutírico, rizogênese.

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## Introduction

Azalea plants (*Rhododendron simsii* Planch.), belonging to the Ericaceae family, is an ornamental plant native to China (Christiaens et al., 2014), but widely used in Brazil due to the beauty of its flower colors and shapes. It is one of the main potted flowering plants marketed in Brazil (Paiva et al., 2016).

This segment presents significant importance in the global market. A recent analysis by the Brazilian Micro and Small Business Support Service (Sebrae, 2015) showed growth in this sector from 20% to 24% from 2008 to 2013. In this same analysis, it was noted that such growth also reflects the behavior of consumers in the international market, which points to the growing importance of flowering and potted plants in the consumption pattern, given the better cost-benefit ratio when compared with cut flowers due to lower relative costs as well as greater durability and convenience of flowering and potted plants.

For the contemporary consumer, these goods are more adequate to the even more restrictive economic environment of the international market, in addition to being more adapted to the current lifestyle, marked by smaller housing and less presence of accessories such as pots for cut flowers and floral arrangements.

Azalea propagation is made commercially by cuttings (Lone et al., 2010), however, its rooting percentage is low, interfering negatively in seedling production.

Several factors affect root initiation and development, both of endogenous and exogenous nature; among them, the hormonal balance in the tissue is indicated as of great importance for rooting (Rosa et al., 2017). Also, boron may have a positive effect, since it acts on the synthesis of proteins and nucleic acids (Cunha et al., 2009).

The plant regulator that acts directly in root induction is the auxin, coming from meristematic regions of the plant. Indolebutyric acid (IBA) is the most widely used synthetic auxin because of its greater stability (Figueiredo et al., 2009). The auxin is a class of plant regulators that promotes rooting of cuttings (Pêgo et al., 2019).

Boron is an important micronutrient for adventitious rooting and it is essential in the phases of induction and development of root primordia and later growth (Cunha et al., 2009). It is considered a co-factor of rooting due to its role in the rhizogenic process together with auxin, facilitating membrane transport and its integrity maintenance (Herrera et al., 2004).

Therefore, studies are needed to maximize rooting of azalea cuttings from cultivars considered important economically and difficult to propagate. Thus, the objective of this study was to evaluate the influence of indolebutyric acid (IBA) and boron on the rooting of herbaceous azalea cuttings of Otto and Terra Nova cultivars.

## Material and methods

The experiment was conducted at the commercial property "Sleutjes Azaleias", specialized in potted azalea cultivation, located in Holambra County, São Paulo State,

Brazil, and the evaluations were carried out at the Plant Seed Laboratory of the Crop Production Department, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo State, from June 10<sup>th</sup> to July 22<sup>nd</sup>, 2016.

The herbaceous cuttings of Otto and Terra Nova cultivars, standardized with  $10 \pm 1$  cm length, were collected from plants cultivated in pots at the production area. Cuttings were maintained with seven to 10 pairs of leaves while the basal leaves were removed according to the company usual procedures. The bases of the cuttings (1/3 of the length), firstly immersed in hydrous solution (50% ethyl alcohol) in the different IBA concentrations, were subsequently treated with solutions of different boron concentrations, which source was boric acid (17% B), according to the treatments, where cuttings were immersed for a similar period to auxin immersion.

After treatment, 40 cuttings were placed per plastic tray (41.5 x 29.5 x 7.5 cm) of 7 L capacity, containing a mixture of 80% pine bark and 20% peat as substrate. Subsequently, a solution of 1.6 mL L<sup>-1</sup> Bravonil® fungicide + 0.2 mL L<sup>-1</sup> Actara® insecticide was applied with a hand irrigator and, soon after, the trays were covered with transparent plastic and kept at a greenhouse for a period of six weeks, as the grower considers appropriate for rooting. The trays were maintained under a shading net, which when closed, promoted up to 730 W m<sup>-2</sup>, with no control over the local humidity, however, a ventilation system was used.

The following evaluations were then performed: rooting percentage, root number, length (cm), volume (cm<sup>3</sup>), surface area (cm<sup>2</sup>), mean diameter (cm), and dry matter (mg).

After counting the number of rooted cuttings, roots were removed for the remaining analysis. Root number, mean and total length, volume, surface area, and mean root diameter were analyzed by photointerpretation using Safira® Software, so roots were photographed along with a length reference, and the images submitted for analysis in the software. Roots were then placed in a forced-air circulation oven for 48 hours at 65 °C and then weighed using an analytical balance.

The statistical design was completely randomized, with 16 treatments arranged in a 4x4 factorial scheme (IBA concentrations: 0; 1,000; 2,000; and 3,000 mg L<sup>-1</sup> x boron concentrations: 0; 2.5; 5.0; and 7.5 mg L<sup>-1</sup>), four replications and 10 cuttings per replication. Variance analyses were performed to verify the significance of the variables studied and the interaction between them. Also, analysis of polynomial regression was conducted to verify variable behavior according to the increase of IBA and boron concentrations, as well as of correlation among variables.

## Results and Discussion

### Statistical analysis of IBA and boron application on Otto and Terra Nova cultivars

Root number, length and dry matter of Otto cuttings were influenced by IBA and the association of IBA with

boron; root surface area and volume were influenced only by the different IBA concentrations (Table 1). However, for rooting percentage and root diameter, there

were no significant differences for this cultivar from both IBA and boron, neither for the interaction between them (Table 1).

**Table 1.** ANOVA analysis for root agronomic variables of azalea cuttings (*Rhododendron simsii* Planch.), Otto cultivar, submitted to different IBA and boron concentrations.

Rooting (%)	GL	SQ	QM	F	CV(%)
IBA	3	380.10	126.70	0.99 <sup>ns</sup>	13.90
Boron	3	390.62	130.20	1.02 <sup>ns</sup>	
IBA x Boron	9	604.63	67.18	0.52 <sup>ns</sup>	
Root Number	GL	SQ	QM	F	CV(%)
IBA	3	9,106.60	3,035.53	15.57**	23.07
Boron	3	447.82	149.27	0.77 <sup>ns</sup>	
IBA x Boron	9	8,719.45	968.82	4.97**	
Root Length (cm root <sup>-1</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	46.24	15.41	10.06**	19.07
Boron	3	0.83	0.27	0.18 <sup>ns</sup>	
IBA x Boron	9	54.62	6.06	3.96**	
Root Dry Matter (mg)	GL	SQ	QM	F	CV(%)
IBA	3	0.00290	0.000968	7.22**	20.58
Boron	3	0.00004	0.000013	0.09 <sup>ns</sup>	
IBA x Boron	9	0.00131	0.000146	1.08**	
Root Surface Area (cm <sup>2</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	2,514.87	838.13	8.44**	25.91
Boron	3	316.87	105.62	1.06 <sup>ns</sup>	
IBA x Boron	9	1,967.44	218.60	2.20 <sup>ns</sup>	
Root Volume (cm <sup>3</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	888.88	296.29	5.48*	33.57
Boron	3	206.39	68.79	1.27 <sup>ns</sup>	
IBA x Boron	9	789.92	87.76	1.62 <sup>ns</sup>	
Root Diameter (cm root <sup>-1</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	0.186	0.062	1.199 <sup>ns</sup>	21.64
Boron	3	0.074	0.024	0.478 <sup>ns</sup>	
IBA x Boron	9	0.535	0.594	1.147 <sup>ns</sup>	

<sup>ns</sup>non significant; \*\*significant at 1% probability; \*significant at 5% probability.

Similarly to what occurred for cv. Otto, root number for 'Terra Nova' was influenced by IBA, boron, and the interaction between them (Table 2). However, there were no significant differences for rooting percentage and root diameter from IBA, boron, or the interaction

between them. The association between the growth regulator and boron also influenced root length of cv. Terra Nova cuttings. These variables, added to root number, are important characteristics indicating the root system quality.

**Table 2.** ANOVA analysis for root agronomic variables of azalea cuttings (*Rhododendron simsii* Planch.), Terra Nova cultivar, submitted to different IBA and boron concentrations.

Rooting (%)	GL	SQ	QM	F	CV(%)
IBA	3	29.68	9.89	1.46 <sup>ns</sup>	2.62
Boron	3	29.68	9.89	1.46 <sup>ns</sup>	
IBA x Boron	9	76.56	8.50	1.26 <sup>ns</sup>	
Root Number	GL	SQ	QM	F	CV(%)
IBA	3	17,811.12	5,937.04	19.54**	16.45
Boron	3	15,868.37	5,289.45	17.41**	
IBA x Boron	9	12,309.75	1,367.75	4.50**	
Root Length (cm root <sup>-1</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	0.15	0.05	0.98 <sup>ns</sup>	18.68
Boron	3	0.13	0.04	0.85 <sup>ns</sup>	
IBA x Boron	9	1.15	0.12	2.53*	
Root Dry Matter (mg)	GL	SQ	QM	F	CV(%)
IBA	3	5.64	1.88	3.04**	25.92
Boron	3	8.23	2.74	4.45**	
IBA x Boron	9	4.08	0.45	0.73 <sup>ns</sup>	
Root Surface Area (cm <sup>2</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	4.94	1.64	0.95 <sup>ns</sup>	22.02
Boron	3	17.30	5.77	3.33**	
IBA x Boron	9	29.06	3.23	1.86 <sup>ns</sup>	
Root Volume (cm <sup>3</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	0.36	0.12	0.38 <sup>ns</sup>	45.59
Boron	3	5.51	1.83	5.80**	
IBA x Boron	9	3.39	0.37	1.19 <sup>ns</sup>	
Root Diameter (cm root <sup>-1</sup> )	GL	SQ	QM	F	CV(%)
IBA	3	0.010	0.003	0.83 <sup>ns</sup>	19.87
Boron	3	0.027	0.009	2.21 <sup>ns</sup>	
IBA x Boron	9	0.041	0.004	1.12 <sup>ns</sup>	

<sup>ns</sup>non significant; \*\*significant at 1% probability; \*significant at 5% probability.

For root dry matter of this same cultivar (Terra Nova), both treatments were significant separately, while root surface area and volume show differences only when cuttings were treated with boron concentrations (Table 2). The absence of significance for rooting percentage for both cultivars (Tables 1 and 2) may be due to the fact that cuttings already had enough endogenous auxins to root, as well as boron, so that root formation would occur anyway. Cutting harvesting time may have favored this result, since matrices had not yet undergone conditions of low temperatures, which naturally decrease plant metabolism as explained by Hartmann et al. (2011). The same authors also support another factor that may have favored this result, which is the leaf presence in the cuttings, since they continue the process of production and supply of carbohydrates used

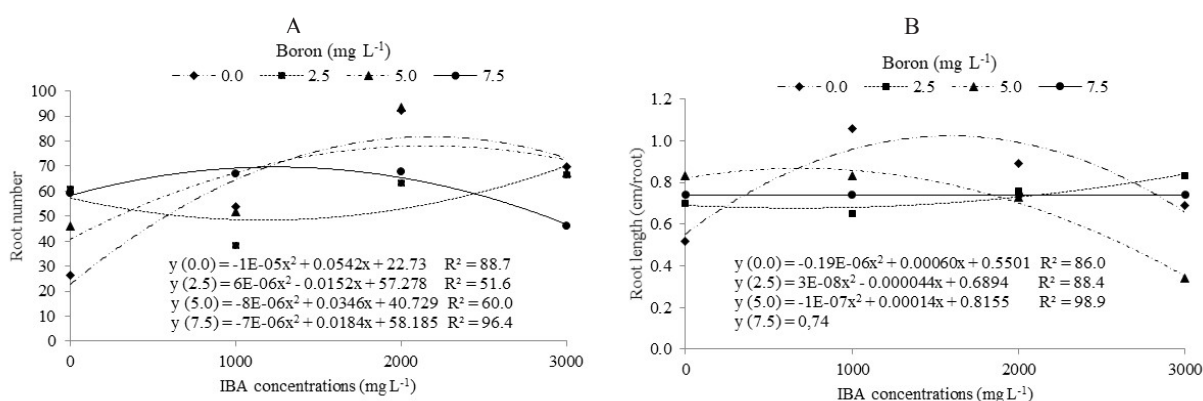
in the rhizogenic process. Similar results were found by Valmorbida and Lessa (2008), who also observed that there was no effect of IBA and boron on rooting percentage of ginkgo tree cuttings (*Ginkgo biloba*). Furthermore, for the same azalea genus, Salvador, Jadoski and Resende (2005) observed that there was no effect of IBA on rooting percentage of *Rhododendron indicum* cuttings. For the same species of the present study, i.e., *R. simsii*, similar results were observed by other researchers. Feliciano et al. (2017), using IBA concentrations of 0; 5,000; 6,000; 7,000; and 8,000 mg L<sup>-1</sup> for 10 seconds, observed that there was no significant difference in rooting percentage. However, cuttings rooted even under IBA absence, so the authors suggested that this fact may be attributed to the presence of endogenous auxins already present in the cuttings. Lone et

al. (2010), using cuttings either treated or not with 1,000 mg L<sup>-1</sup> IBA, placed in different substrates, also observed that there was no IBA effect on rooting percentage. However, in both studies, the authors did not cite the cultivar used, probably an old one used in gardens, which may be of easy rooting. The time of cutting harvesting may also have favored such result since, for both surveys, cuttings were performed in the beginning of fall. However, Feliciano et al. (2017), still studying rooting of this same species with different cutting length, verified that 10-cm length cuttings presented better rooting when compared with those of 12 and 15 cm length. The authors suggested that larger cuttings presented greater loss of water, impairing rooting. When using naphthalene acetic acid (NAA) at

the concentrations of 0; 2,000; 4,000; 6,000; and 8,000 mg L<sup>-1</sup>, these authors verified that its application reduced rooting percentage of azalea cuttings by assigning that the amount used was toxic.

#### Effect of IBA and boron on Otto cultivar

There was a significant interaction between IBA and boron for root number of Otto azalea cuttings (Table 1). Higher means were observed either in the absence of boron or 5.0 mg L<sup>-1</sup> combined with 2,000 mg L<sup>-1</sup> IBA. When there was no IBA addition, those cuttings treated with boron in the concentrations of 2.5; 5.0; and 7.5 mg L<sup>-1</sup> showed higher root number when compared with 0 mg L<sup>-1</sup> boron (Figure 1A).



**Figure 1.** Root number (A) and length (B) of azalea cuttings (*Rhododendron simsii* Planch.), Otto cultivar, submitted to different IBA and boron concentrations.

When boron-treated Otto cuttings are observed, root number increases under 2,000 mg L<sup>-1</sup> IBA (Figure 1A), demonstrating the action of the exogenous auxin stimulating root formation and number (Figueiredo et al., 2009, Taiz et al., 2017). However, from this concentration, there is a tendency to reduce root number, indicating phytotoxicity when the concentration increases (Figure 1A). In addition, it is important to consider the ability of some plants to mobilize boron from mature leaves to young tissues, as it occurs in some eucalyptus (*Eucalyptus* spp.) genotypes (Mattiello et al., 2009), and may influence the process of rooting since this fact occurs mainly in boron-deficient plants. In this sense, it is thought that in a cutting where boron can be mobilized, it will be taken from an older tissue to the region where it is demanding, that is, for the development of adventitious roots.

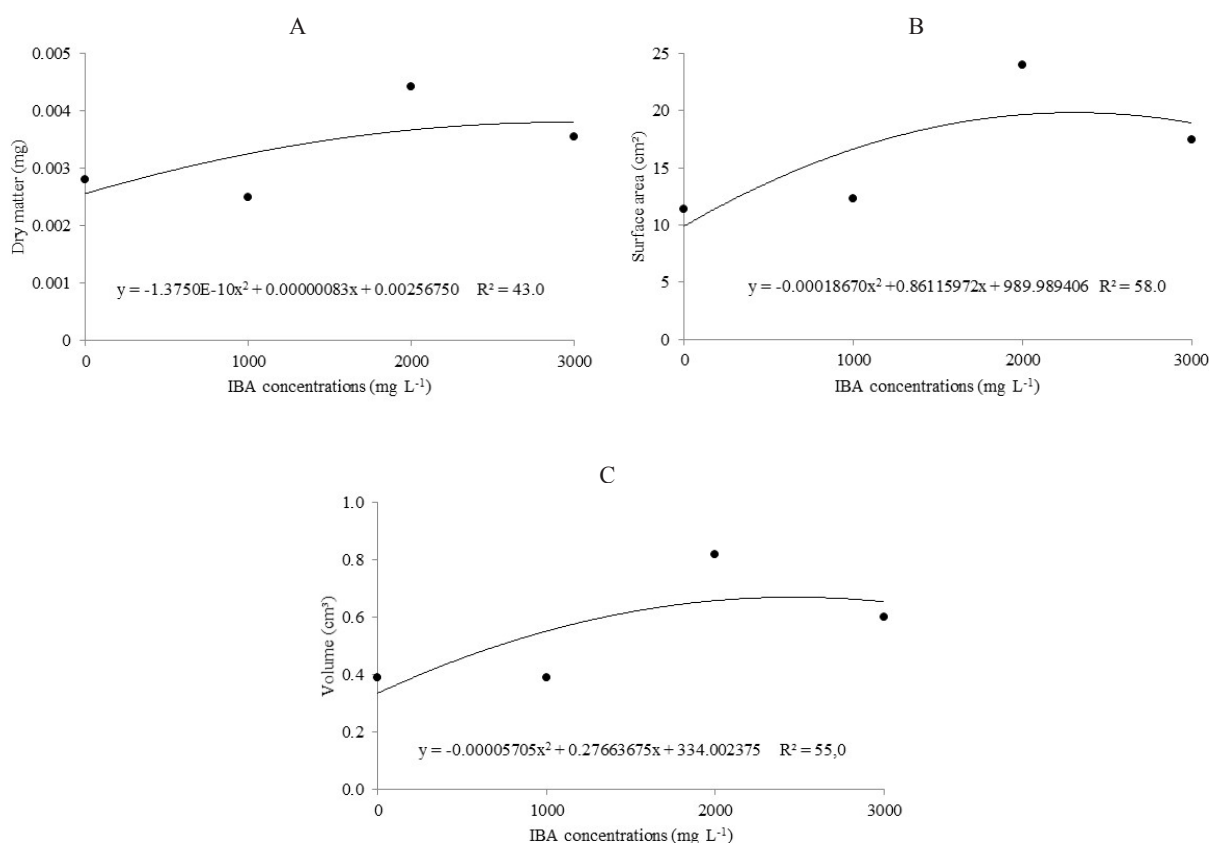
According to Figure 1A, all treatments were superior to the control (0 mg L<sup>-1</sup> IBA and 0 mg L<sup>-1</sup> boron). The highest number of roots for cuttings of Otto cultivar was observed at the concentration of 2,000 mg L<sup>-1</sup> IBA with no boron addition. However, combinations of 7.5 mg L<sup>-1</sup> boron with 0 or 1,000 mg L<sup>-1</sup> IBA, 0 or 5.0 mg L<sup>-1</sup> boron plus 2,000 mg L<sup>-1</sup> IBA, and 2.5 mg L<sup>-1</sup> boron with 3,000 mg L<sup>-1</sup> IBA were also the arrangements showing satisfactory results for this variable, suggesting that, for practical purposes, one can opt for the combination that results in greater savings

of these inputs for seedling production. Similarly, for root length of Otto cultivar, higher means were observed at the concentrations of 1,000 and 2,000 mg L<sup>-1</sup> IBA in the absence of boron (Figure 1B). Conversely, studying another cultivar of the same species, Lone et al. (2010) did not observe a significant difference for the variable length of the largest root in either treated or non-treated cuttings with 1,000 mg L<sup>-1</sup> IBA. Paulus et al. (2016), when studying different IBA concentrations and cutting harvesting time in the vegetative propagation of rosemary apical cuttings (*Rosmarinus officinalis*), observed that the use of IBA favored both rooting and root length, showing also that there is no interference from the seasons in the vegetative propagation of this species.

The root length of Otto (Figure 1B), with the exception of the boron 7.5 mg L<sup>-1</sup> concentration, was not significant from the association with any of the IBA concentrations studied, that is, independently of the IBA addition, it will not influence the result found for root length of azalea cuttings treated with 7.5 mg L<sup>-1</sup> boron. Although the highest mean number of roots were verified in the absence of boron with 2,000 mg L<sup>-1</sup> IBA, any of the boron concentrations tested plus IBA 2,000 mg L<sup>-1</sup> (Figure 1B) showed a better result than the control, that is, 0 mg L<sup>-1</sup> IBA and 0 mg L<sup>-1</sup> boron. Thus, in general, the combination of boron and auxin benefited the increase in root length of Otto azalea cuttings;

however, highest root length means were obtained in the absence of this micronutrient. Salibe et al. (2010) verified that boric acid had few effects on the variables related to grapevine rooting, and that only the IBA application increased rooting. Similar results occurred in bamboo orchid (*Arundina bambusifolia*) treated or non-treated with boron and IBA concentrations; the treatments with boric acid were not efficient, so 400 mg mL<sup>-1</sup> IBA was the treatment that induced a higher percentage of shoots and rooted shoots (Mengarda et al., 2013). However, boron has been shown to be important in the root initiation stage, as well as in processes of growth and development of the root system such as cell wall formation, lignification, and

elongation, besides being necessary in the development of root primordia and its subsequent growth (Cunha et al., 2009). IBA also influenced root length of yellow jasmine (*Jasminum mesnyi*) cuttings (Fanti et al., 2013). In addition, 8,000 mg L<sup>-1</sup> IBA promoted greater number of longer roots (Oliveira et al., 2015). For root dry matter of Otto cuttings (Figure 2A), there was significance only for IBA, since the use of boron did not affect the results for this variable, and no interaction was observed among IBA and boron concentrations. Similarly to that observed for root number and length (Figure 1), higher means were observed when 2,000 mg L<sup>-1</sup> IBA was applied (Figure 2A).



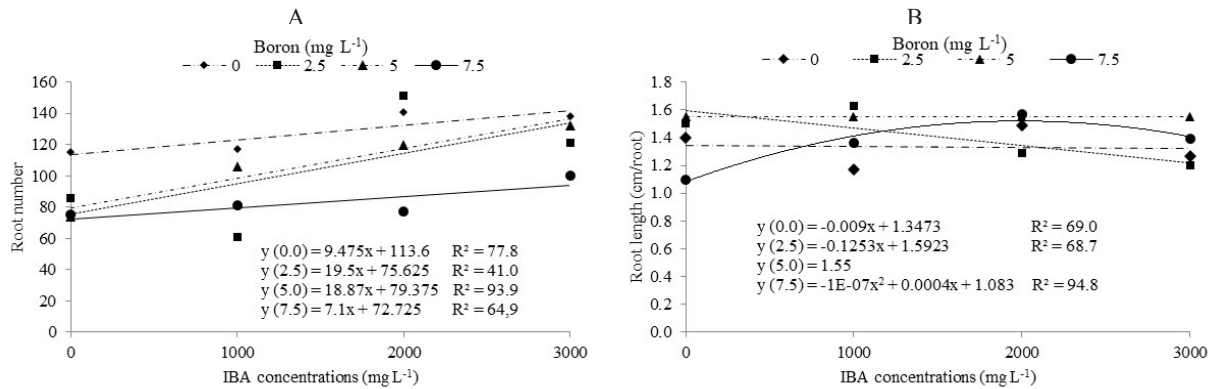
**Figure 2.** Root dry matter (A), surface area (B), and volume (C) of azalea cuttings (*Rhododendron simsii* Planch.), Otto cultivar, submitted to different IBA concentrations.

For some species, the effect of boron on root dry matter has been observed. Araújo et al. (2017), when studying *Khaya senegalensis* seedlings, verified a reduction in dry matter of stems, roots and leaves, as well as in height, stem diameter and leaflet number according to the increment in boron doses, what suggests there is a fine line for crop use and it becomes toxic in high concentrations. However, there was a 72% increase in shoot dry matter of watermelon cv. Crisson Sweet (*Citrullus lanatus*) when 10.5 g B Kg<sup>-1</sup> of seeds were used (Silva-Matos et al., 2017), which was not observed in this work with boron addition for cutting treatment. According to Xavier and Natale (2017), when studying star fruit trees (*Averrhoa carambola*), the increase of boron concentrations decreased both boron and calcium levels in the roots, as well as the efficiency of

transporting these elements from roots to the plant aerial part. The root dry matter of Otto azalea cuttings submitted to increasing IBA concentrations showed a significant rise up to 2,000 mg L<sup>-1</sup> (Figure 2A), with an increment of 43.4% from the lowest to the highest concentration of the plant regulator.

#### Effect of IBA and boron on Terra Nova cultivar

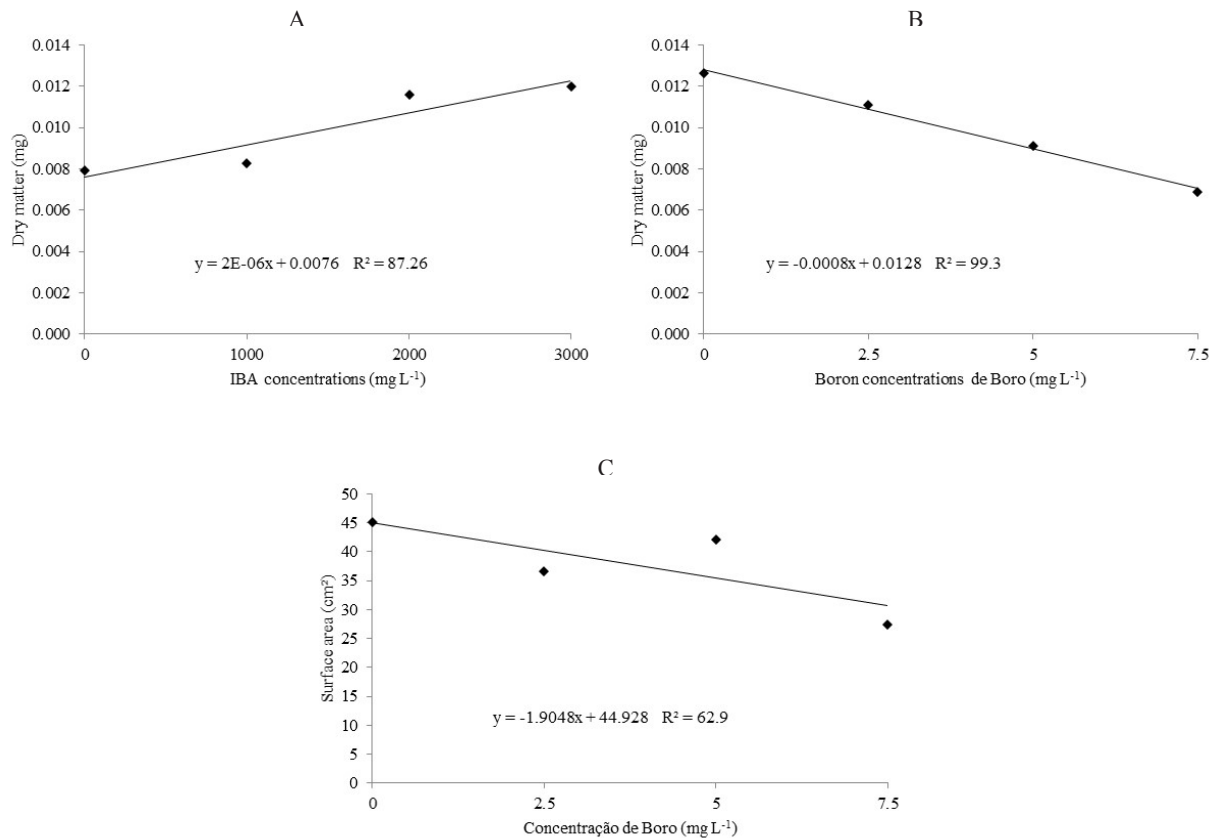
In general, for Terra Nova cuttings, the root number increased with the addition of any IBA concentration (Figure 3A). However, when cuttings were not treated with IBA or boron (0 mg L<sup>-1</sup> IBA and 0 mg L<sup>-1</sup> boron), they had 115 roots, a higher number compared with 2.5, 5.0, and 7.5 mg L<sup>-1</sup> boron concentrations, which resulted in 86, 74, and 75 roots, respectively.



**Figure 3.** Root number (A) and length (B) of azalea cuttings (*Rhododendron simsii* Planch.), Terra Nova cultivar, submitted to different IBA and boron concentrations.

The concentration of 3,000 mg L<sup>-1</sup> IBA with no boron was the ratio that presented the highest number of roots formed by cutting. Thus, it can be stated that Terra Nova cultivar is more resistant to high concentrations of IBA and boron when compared with Otto, because from 3,000 mg L<sup>-1</sup> IBA with 0 mg L<sup>-1</sup> boron, Terra Nova cuttings presented an increasing tendency for the variable root number (Figure 3A). The behavior of the variable root length did not follow the same increment trend as root number, suggesting that small roots were formed, only in greater quantity (Figure 3B). According to this result, Lima et al. (2018) affirm that it is important to have more roots than longer ones, since cuttings with larger roots are more likely to impair transplanting. In this way, the highest number of physiologically active roots concomitantly with the largest surface area reflected in a larger portion of soil explored, producing positive effects on nutrient uptake (Borcioni et al., 2016). It is still observed that those Terra Nova cuttings that did not receive boron as the treatment, showed a subtle reduction in root length (Figure 3B), from 1.4 cm to 1.3 cm with the addition of 3,000 mg L<sup>-1</sup> IBA. The most significant difference was from 2.5 mg L<sup>-1</sup> boron with IBA concentrations; while initially the roots formed 1.5 cm in length, at the end of the highest IBA concentration, that is 3,000 mg L<sup>-1</sup>, roots were 1.2 cm long, resulting in a 20% reduction in length (Figure 3B). On the other hand, plants receiving the highest

boron concentration behaved differently from the other concentrations tested, with an increase in root length from the association of 7.5 mg L<sup>-1</sup> boron with 2,000 mg L<sup>-1</sup> IBA; from this IBA concentration, the variable had a tendency to decrease (Figure 3B). The association of boron with auxin may have a synergistic effect, as it positively influenced the development of roots in vine cuttings (*Vitis vinifera*) (Jarvis et al., 1983). Boron may then be considered a cofactor of rooting, relating synergistically with IBA and increasing the success of root emission (Hirsch and Torrey, 1980). In general, it was observed that, at the concentration of 2,000 mg L<sup>-1</sup> IBA, the highest means for root number, mean and total length, dry matter, surface area, and volume were verified, indicating better quality of the root system of Terra Nova azalea cultivar, which improves plant support and increases the chance of plant survival, thus resulting in more vigorous and better quality seedlings. The root dry matter of Terra Nova cuttings was not influenced by the interaction between IBA and boron (Figure 4). The addition of different IBA concentrations was beneficial to the cuttings, represented by the linear regression increasing 48.9%, that is, the higher the IBA concentration, the greater the root dry matter formed by Terra Nova azalea cuttings (Figure 4A). On the other hand, boron impaired root dry matter increment, reducing 45.6% of the initial root matter from cuttings that were not treated with the micronutrient (Figure 4B).



**Figure 4.** Root dry matter of azalea cuttings (*Rhododendron simsii* Planch.), Terra Nova cultivar, submitted to different concentrations of IBA (A) and boron (B), and root surface area (C) of cuttings submitted to different boron concentrations.

The results of the root surface area for this same cultivar were significant only for different boron concentrations, that is, only boron affected the results of this variable (Figure 4C). The increase of any concentration was enough to reduce the surface area, but at 7.5 mg L<sup>-1</sup> boron, there was a decrease of 39.2% in relation to the boron absence in the cuttings. According to the results, it is verified that Terra Nova cultivar has, probably, enough boron in the cuttings to aid in rooting and that the addition of this micronutrient may damage the root system.

#### Polynomial regression and correlation analysis for Otto and Terra Nova cultivars

From the regression analysis for the variables root surface area and volume of Otto cultivar, it was also observed that higher means were found when 2,000 mg L<sup>-1</sup> IBA was used (Figures 2B and 2C). In the rooting process, there is a sequence of events that occur concomitantly and interconnected. The correlation among the studied variables evidences such rooting process (Tables 3 and 4). It is known that auxin and boron are closely linked in rhizogenesis, auxin by root induction and boron by the action of cell elongation, nucleic acid synthesis and membrane function (Taiz et al., 2017).



**Table 3.** Correlation among variables studied for the influence of IBA and boron on azalea cuttings (*Rhododendron simsii* Planch.), Otto cultivar: rooting (ROO); root number (RN); root length (RL); root dry matter (RDM); root volume (RV); root surface area (RSA); and root mean diameter (RMD).

	ROO	RN	RL	RDM	RV	RSA	RMD
ROO	1	0.29*	-0.09 <sup>ns</sup>	0.33**	0.13 <sup>ns</sup>	0.20 <sup>ns</sup>	-0.03 <sup>ns</sup>
RN		1	-0.01 <sup>ns</sup>	0.76**	0.62**	0.75**	0.20 <sup>ns</sup>
RL			1	0.18 <sup>ns</sup>	0.37**	0.33**	0.40**
RDM				1	0.55**	0.65**	0.21 <sup>ns</sup>
RV					1	0.97**	0.53**
RSA						1	0.48**
RMD							1

<sup>ns</sup>non significant; \*\*significant at 1% probability; \*significant at 5% probability.

**Table 4.** Influence among the variables studied in the rooting process of azalea cuttings (*Rhododendron simsii* Planch.), Otto cultivar, represented by the simple correlation among variables: root number (RN); root length (RL); root dry matter (RDM); root volume (RV); root surface area (RSA); and root mean diameter (RMD).

Influence among root variables										
RN	→	RV	→	RSA						
		RV	→	RSA	→	RMD				
				RSA	→	RMD	→	RL		
								RL	→	RDM
										RDM → NR

In this experiment, boron had no effective action on root formation of Otto cuttings, certainly because there is enough quantity in the cuttings. However, the exogenous auxin was able to directly influence root number, mean and total length, dry matter, surface area, and volume. In Table 4, it is possible to understand how one variable affects the other in a cascade effect, for example, 2,000 mg L<sup>-1</sup> IBA is able to increase root number; in this way, root number has a direct correlation with root volume and area, that is, if there is an increase in root number, there is also an increment in root volume and surface area of Otto cuttings. In turn, the increment in root volume and surface area will increase root mean diameter, which will increase mean and total root length, which will add in root dry matter. The increase in root dry matter has a direct correlation with root number and, the latter, with rooting. It is noted that IBA was not able to directly increase rooting, but that, by a cascade effect, indirectly influenced rooting success of Otto azalea cuttings. Castro et al. (2012) report that boron reacts with sugars to form an ionizable borate-sugar

complex capable of passing through cell membranes faster than non-ionized sugar molecules without borate, being translocated with greater agility to growth cells, in this case the developing roots. As there was no response to the addition of boron, it is assumed that there were already necessary quantities for root formation. Thus, with regard to the improvement in quality of Otto azalea seedlings, the application of 2,000 mg L<sup>-1</sup> IBA on cuttings already favors the development of a root system with higher root number, mean and total length, dry matter, surface area, and volume, since seedlings with well-developed roots have greater ability to explore the substrate, guaranteeing the development of a plant with greater vigor, reducing the production cycle and allowing greater survival. Values of the correlation coefficients among the variables analyzed for Terra Nova azalea cuttings are presented in Table 5. Positive correlations were observed between rooting percentage and root dry matter. Root number was correlated with root total length, dry matter, volume, surface area, and diameter.

**Table 5.** Correlation among variables studied for the influence of IBA and boron on azalea rooting (*Rhododendron simsii* Planch.), Terra Nova cultivar: rooting (ROO); root number (RN); root length (RL); root dry matter (RDM); root volume (RV); root surface area (RSA); and root mean diameter (RMD).

	ROO	RN	RL	RDM	RV	RSA	RMD
ROO	1	0.23 <sup>ns</sup>	0.06 <sup>ns</sup>	0.31*	0.13 <sup>ns</sup>	0.09 <sup>ns</sup>	0.18 <sup>ns</sup>
RN		1	-0.06 <sup>ns</sup>	0.67**	0.43**	0.31*	0.55**
RL			1	0.02 <sup>ns</sup>	0.11 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.12 <sup>ns</sup>
RDM				1	0.33**	0.24 <sup>ns</sup>	0.52**
RV					1	0.23 <sup>ns</sup>	0.60**
RSA						1	0.12 <sup>ns</sup>
RMD							1

<sup>ns</sup>non significant; \*\*significant at 1% probability; \*significant at 5% probability.

The high correlation observed between root number and root diameter ( $r = 0.55$ ) for Terra Nova cultivar allows to affirm that the more roots formed by cutting, the larger the diameter; thus, higher volume accounting for the higher content of dry matter. Therefore, the dry matter may be used in the root system evaluation for this cultivar, since it obtained a high correlation with root number ( $r = 0.67$ ), as the more roots formed per cutting, the greater the chance of success of seedling formation (Table 5). The addition of IBA and boron to the base of the cuttings had no effect on rooting percentage; however, it modified the root system formed. Furthermore, IBA was beneficial to root dry mat-

ter increment while boron had a detrimental effect on both root dry matter and surface area. This allows to note that the results of root number and length, with the addition of only IBA concentrations, were superior to all other combinations, reinforcing that this cultivar already has enough boron and its increase may cause a reduced root number as well as shorter roots, resulting in lower dry matter and surface area. According to Table 6, the IBA application did not directly influence rooting, but improved the root system formed by a chain reaction, i.e., there was an increase in root number, which, in turn, increased root mean and total length.

**Table 6.** Influence among the variables studied in the rooting process of azalea cuttings (*Rhododendron simsii* Planch.), Terra Nova cultivar, represented by the simple correlation among variables: rooting (ROO); root number (RN); root length (RL); root dry matter (RDM) and root volume (RV).

Influence among variables				
RN	→	RL		
RN	→	RL	→	RV
				RV → RDM
				RDM → ROO

The increase in root total length increases the root system volume, which directly increases root dry matter, contributing to rooting success, thus ensuring the formation of seedlings with a more vigorous root system.

## Conclusions

For the azalea cultivars 'Otto' and 'Terra Nova', IBA and boron concentrations did not influence rooting percentage. Root number, mean and total length, dry matter, surface area, and volume presented positive results to the application of 2,000 mg L<sup>-1</sup> IBA, in the absence of

boron, for Otto azalea cuttings. Root dry matter and surface area were impaired by the addition of boron, but there was a higher number of roots with the application of 3,000 mg L<sup>-1</sup> IBA; however, this concentration affected root length of Terra Nova azalea cuttings.

## Author Contribution

**A.K.D.B.** <sup>0000-0002-6624-5364</sup>: conception and design of the research, obtaining data, analysis and interpretation of data, statistical analysis, writing and critical analysis of the manuscript. **M.V.F.** <sup>0000-0002-5940-8196</sup>: analysis and interpretation of data, writing

and critical analysis of the manuscript. **K.F.L.P.** <sup>0000-0001-9983-2402</sup>; conception and design of the research, obtaining data, analysis and interpretation of data, statistical analysis, writing and critical analysis of the manuscript. **M.R.N.** <sup>0000-0002-6484-0354</sup>; analysis and interpretation of data, writing and critical analysis of the manuscript. **R.B.M.G.** <sup>0000-0003-2424-4862</sup>; analysis and interpretation of data, writing and critical analysis of the manuscript.

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