Schwartz, G., da Silva Rocha, T., Sá de ALmeida, M. J., Silva Dionisio, L. F., Corvera, R. (2022): Seedlings quality of Bertholletia excelsa Bonpl. (Lecythidaceae) produced in forest nursery, Agriculture and Forestry, 68 (1): 219-232. doi:10.17707/AgricultForest.68.1.14

DOI: 10.17707/AgricultForest.68.1.14

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SEEDLINGS QUALITY OF *BERTHOLLETIA EXCELSA* BONPL. (LECYTHIDACEAE) PRODUCED IN FOREST NURSERY

SUMMARY

In this study, the quality of *Bertholletia excelsa* seedlings was evaluated in relation to the transplanted seedling's size. Thirty days after transplanting the seedlings to the forest nursery, the first height measurement was performed. From the 30th day onwards, evaluations were carried out every 15 days, being nine in total. The Dickson Quality Index (DQI) was evaluated to determine the seedlings quality, where all seedlings had their root pruned. The experimental design, totaling 160 individuals, was completely randomized with four treatments and four replications, including the shoot cut and three seedling sizes with no shoot cut. Shoot cut seedlings presented a DQI of 0.66 ± 0.23, significantly higher than the large seedlings (≤ 17 cm in height) with no shoot cut ($F_{3,76}$ = 2.762, p = 0.047). Transplanting had significant effects over the development, growth, and quality of *Bertholletia excelsa* seedlings during the first 165 days of seedling production. Therefore, shoot pruning resulted in better performance of *B. excelsa* seedlings to be planted in the field.

Keywords: Brazil nut, seedling transplanting, seedling production, native tree species, Peruvian Amazon

INTRODUCTION

Bertholletia excelsa Bonpl., commonly known as Brazil nut, is a tree species of the family Lecythidaceae with great economic importance in the

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:04/07/2021 *Accepted:01/02/2022*

Amazonian region (Albuquerque et al. 2015; Nogueira et al. 2018). Its nuts are a non-timber forest product that brings employment and income for thousands of workers in Bolivia, Brazil, and Peru through the seed's market (Scoles et al. 2016, Costa et al. 2015). B. excelsa seeds have high nutritional value and normally attain high commercial value in domestic and international markets (Baldoni et al. 2017, Wadt et al. 2018).

Bertholletia excelsa is a climax and light-demanding species that presents good performance in growth when planted in open areas (Scoles et al. 2011, Albuquerque et al. 2015). Adult trees present large size, reaching 40-60 m in height and 1-4 m in diameter (Santos et al. 2006). In natural environments, B. excelsa tend to present local high densities, because the individuals tend to be aggregated (Mori and Prance 1990).

Seedlings production of tree species is an essential activity to build forest stands, as seedling quality influences the tree development in the field (Dionisio et al. 2019a). Seedling survival under field conditions is a crucial phase in planting, which can be determined by how the seedlings were produced in forest nurseries (Dionisio et al. 2020). The need to produce seedlings both for forest restoration and for commercial purposes requires knowledge, skills, and sensitivity from nurserymen to seek techniques to produce high quality seedlings under low costs (Dionisio et al. 2019b). In this case, seedlings must have characteristics that support the maximum survival rate and rapid initial growth after planting (Auca et al. 2018, Souza et al. 2019).

Currently, there are few recommendations and prescriptions about nursery cultivation of native tree species of the Amazon, as well as the use of alternative containers and substrates that can provide better growth and quality for B. excelsa seedlings. One of the hurdles faced during the process of seedling production of Amazonian native tree species is that many of them are slow-growing species. Furthermore, suitable containers and substrates are also a key to attain high quality seedlings produced in forest nurseries. However, a cultivation system for B. excelsa has not yet been defined, which demands the development of new technologies for that (Auca et al. 2018). Among the factors that influence the production process of tree species seedlings, there are: kind of substrate, substrate moisture, substrate porosity, seeds dormancy, temperature, shading and container volume, irrigation, seed quality, fertilization, and seedling management in the nursery (Camargo et al. 2011, Costa et al. 2015, Marques et al. 2018).

Regarding cultivation practices to optimize seedling production, the benefits of the transplanting process with pruning can be attained with more robust seedlings and better development balance in height and root system. As for the containers, acquisition cost, durability, easy handling, storage, transport, and availability in the market must be considered in seedling production of native tree species. The container's size should be chosen to provide the largest possible volume of substrate to the roots, but with low weight for an easier transport to the field. In this sense, associating nutritional power with the use of smaller containers can be a way to reduce production cost, transport, and field distribution, providing greater efficiency in planting operations (Pinho et al. 2018, Lima Filho et al. 2019).

Currently, there are almost no studies on transplanting methods and techniques to improve seedling quality of B. excelsa. Thus, the objective of this study was to test the initial growth and quality B. excelsa seedlings of different sizes in forest nursery.

MATERIAL AND METHODS

The experiment was carried out in the forest nursery of the Peruvian Amazon Research Institute (IIAP), located in the Department of Madre de Dios, Peru (12º39’04” S, 69º19’17” W) between June and September 2018. Nuts of *Bertholletia excelsa* were collected from the ground around their mother trees in forest concessions of the Peruvian Amazon and manually processed with a machete.

Figure 1. Treatments of shoot pruning (T1); small size individuals, ≤ 5 cm in height (T2); medium size individuals, ≤ 11 cm in height (T3); and large size individuals, ≤ 17 cm in height, (T4) used to assess initial growth and quality of *Bertholletia excelsa* seedligns. All seedlings had root pruned.

Table 1. Treatments used to assess initial growth and quality of *Bertholletia excelsa* seedligns during 165 days in forest nursery, Puerto Maldonado, Peru, 2018.

Treatments	**Repetitions/individuals**
Shoot cut (T1)	4/10
Small individuals (≤ 5 cm in height)/ no shoot cut (T2)	4/10
Medium individuals (≤ 11 cm in height)/ no shoot cut (T3)	4/10
Large individuals (≤ 17 cm in height)/ no shoot cut (T4)	4/10

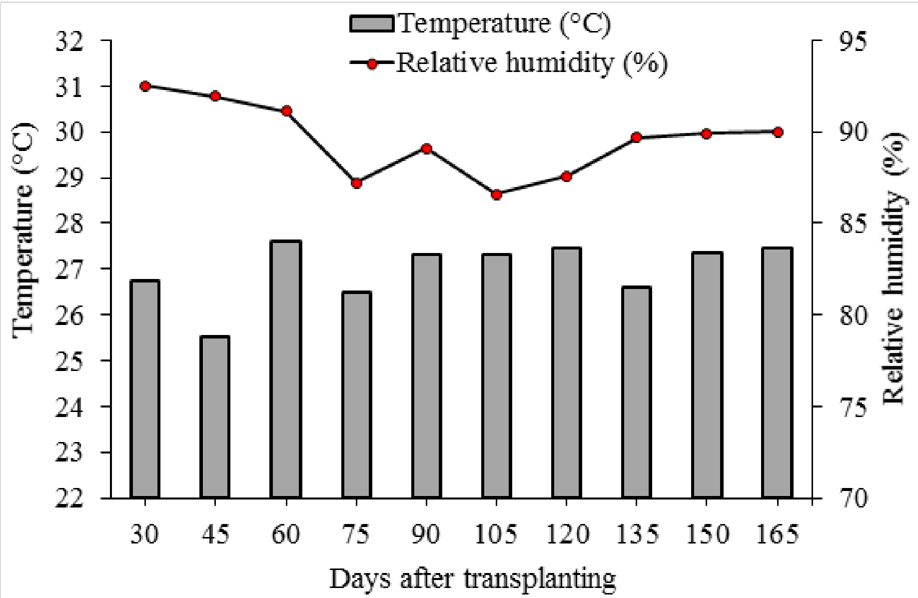

Figure 2. Average values of temperature and relative humidity during the 165 days of the experiment evaluation, Puerto Maldonado, Peru, 2018.

Seeds were immersed in water for 30 days to remove woody integument, and then a lathe was used to remove the bark. Almonds were treated with fungicide Vitavax 300 for 2 h and dried under shade during 1 h. A masonry seedbed measuring 10 m x 1 m x 0.5 m (length, width, and height) was used for sowing. Washed sand was used as substrate for seed germination in the seedbed. The sand was disinfected with boiled water (100 ºC) in 200-liter drums, and 24 h after disinfestation, almonds were sowed. The seedbed was covered with 60% shading mesh at 1 m in height and irrigation was carried out twice a day (morning and afternoon).

A total of 160 *B. excelsa* seedlings had their roots pruned and were transplanted. Besides root pruning of all individuals, the seedling were divided in four treatments as described in Figure 1 and Table 1. Each treatment had four repetitions of 10 seedlings per treatment (Table 1). To evaluate the initial growth, *B. excelsa* seedlings were selected 30 days after germination and transplanted into 115-cm^3 tubes with substrate composed of sand, sawdust, and carbonized sawdust in proportions (1:1:1 v/v). Plants were previously standardized by size and treatments were subsequently established (Table 1).

Thirty days after transplanting seedlings to the forest nursery, the first length measurement was performed. From the 30th day onwards, evaluations were carried out every 15 days, totaling nine evaluations (45, 60, 75, 90, 105, 120, 135, 150, and 165 days). A Data Logger was used for daily measurement of temperature and humidity during the experiment evaluation. Eight daily measurements were performed (1-h intervals between each measurement), where the averages are shown for every two weeks in Figure 2.

Initial seedling growth and seedling quality indexes

Seedlings initial growth was evaluated through the following variables: a) shoot length (SL), b) root length (RL), c) stem collar diameter, d) root collar diameter, e) shoot dry mass (SDM), f) root dry mass (RDM), g) total dry mass (TDM), h) shoot length/root length (SL/RL) ratio, i) shoot dry mass/root dry mass (SDM/RDM) ratio, j) robustness index (RI), k) lignification index (LI), and m) the Dickson Quality Index (DQI). The higher is DQI, RI, and LI, the greater is seedling quality. To measure the stem collar diameter, a digital caliper (accuracy = 0.01 mm) was used and to measure the shoot length and root length, a ruler graduated in millimeters was used.

To assess the seedlings dry mass, the individuals were split into shoots and roots by cutting at the point of the stem collar diameter. Both parts were placed in Kraft paper bags, identified and dried in oven at 70 °C for 72 hours until reaching constant mass. Immediately after the oven, they were weighed on an analytical balance (accuracy = 0.001 g) to obtain the shoot and root dry mass.

The shoot length/root length (SL/RL) ratio predicts the seedling success in a balance between the plant's shoot and the root system. It is determined by the following equation:

$$SL/RL = \frac{Shoot\ length\ (cm)}{Root\ length\ (cm)}$$

The ratio between shoot dry mass/root dry mass (SDM/RDM) indicates the seedling development in nurseries. It is given by the equation:

$$SDM/RDM = \frac{Shoot\ dry\ mass\ (g)}{Root\ dry\ mass\ (g)}$$

The robustness index (RI) was calculated as the ratio between the shoot length and root collar diameter, as follows:

$$RI = \frac{Shoot\ length\ (cm)}{Root\ collar\ diameter\ (mm)}$$

The lignification index (LI) relates the total dry mass with the total wet mass, giving the percentage of lignification. It is determined by the equation:

$$LI = \left(\frac{Total\ dry\ mass\ (g)}{Total\ wet\ mass\ (g)}\right)\ x\ 100$$

The Dickson Quality Index (DQI) brings together several morphological characteristics into a single value given by the following equation:

$$DQI = \frac{Total\ dry\ mass\ (g)}{\frac{Shoot\ length\ (cm)}{Stem\ collar\ diameter\ (mm)}\ \frac{Shoot\ dry\ mass\ (g)}{Root\ dry\ mass\ (g)}}$$

Data analysis

The experimental design applied was completely randomized with four treatments and four replicates of 10 seedlings each (Table 1). To verify the assumptions of the analysis of variance (ANOVA), the data were first checked for: a) normality with the Shapiro-Wilk test ($p > 0.05$), b) homoscedasticity by the Bartlett test ($p > 0.05$), and the independence between experimental units.

Cumulative and relative growth rate during 165 days were submitted to ANOVA and subsequent regression analysis, adjusting the equations to the data obtained as a function of time after transplantation in each treatment. The model for each variable was selected considering the significance of the coefficients of the variables and the highest coefficient of determination (R^2). Tukey's post-hoc test was used to test the regression coefficients ($p < 0.05$). Once these assumptions were met, the data were submitted to ANOVA using the software R, version 3.5.2 and, in case of significant differences, averages were compared by Tukey's post-hoc test ($p < 0.05$).

RESULTS

A higher initial cumulative growth in height of *Bertholletia excelsa* seedlings was observed in the treatment of medium (27.6 cm) and large size individuals (27.7 cm) (Figure 3A). Regarding the relative growth rate (Figure 3B), the shoot cut treatment presented higher cumulative growth, which decreased over time. The treatments small, medium, and large size individuals obtained lower relative growth rate, respectively (Figure 3).

Medium (31.3 ± 4.2 cm) and large (30.7 ± 2.8 cm) individuals had the highest means for shoot length, differing significantly from the other treatments ($F_{3,76} = 19.55$, $p = 0.001$), however, they did not differ significantly ($p = 0.923$) each other (Figure 4A). Regarding root length, the treatment shoot cut had the highest mean (14.1 ± 0.7 cm; $F_{3,\ 76} = 5.621$, $p = 0.002$). Small, medium, and large individuals showed no significant difference among them in root length (Figure 4B). Shoot cut individuals presented the largest stem collar diameter (6.0 ± 1.0; Figure 4C) and in root collar diameter (Figure 4D).

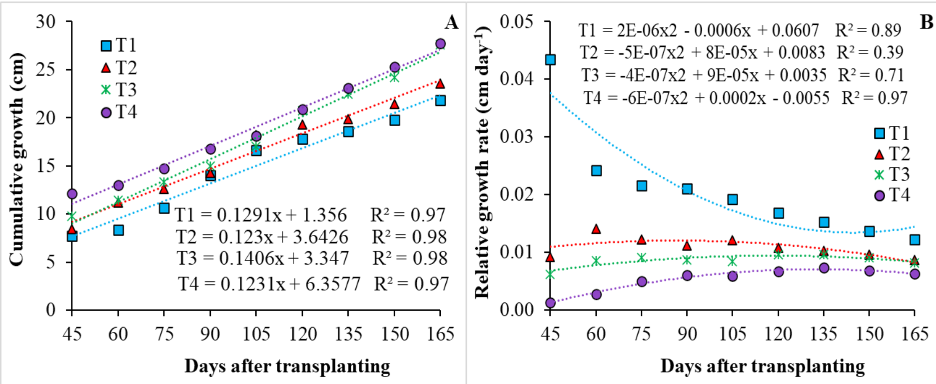

Figure 3. Cumulative growth (A) and relative growth rate in height (B) of *Bertholletia excelsa* seedlings under different sizes as a function of time after root cut and transplanting. Shoot pruning (T1); small size individuals, ≤ 5 cm in height (T2); medium size individuals, ≤ 11 cm in height (T3); and large size individuals, ≤ 17 cm in height, (T4).

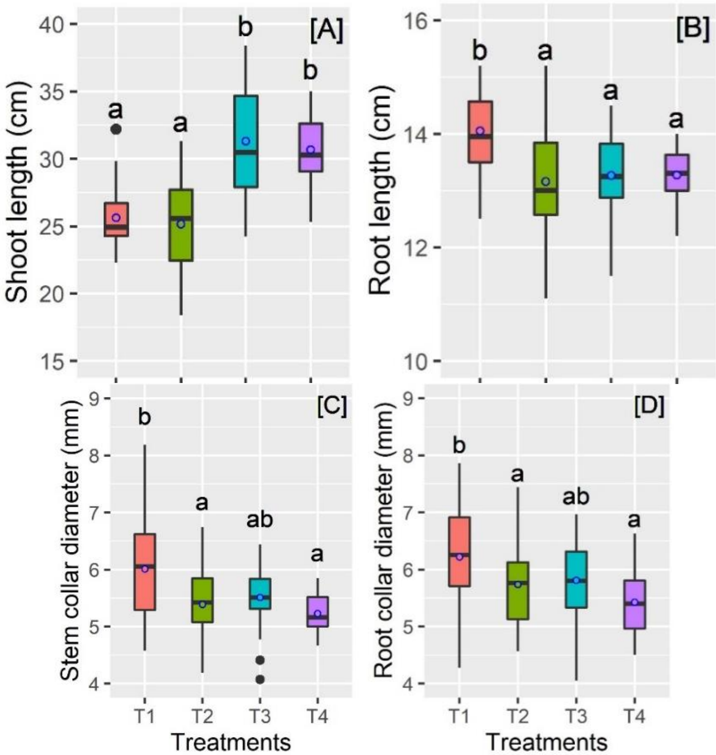

Figure 4. Shoot length (A), root length (B), stem collar diameter (C), and root collar diameter (D) of *Bertholletia excelsa* seedlings under different sizes after root cut and transplanting. Shoot pruning (T1); small size individuals, ≤ 5 cm in height (T2); medium size individuals, ≤ 11 cm in height (T3); and large size individuals, ≤ 17 cm in height, (T4). The thicker horizontal line represents average, the box the interquartile range and the dashed lines the extreme values. Letters indicate statistical differences (p = 0.05) in ANOVA with Tukey's post-hoc test.

The variables shoot dry mass, root dry mass, and total dry mass did not present significant differences among treatments (Figure 5). The shoot length/root length (SL/RL) ratio presented highest averages in medium (2.4 ± 0.3) and large (2.3 ± 0.2) individuals, which differed significantly from treatments shoot cut and small individuals (p = 0.001) (Figure 5D). The shoot dry mass/root dry mass (SDM/RDM) ratio showed no significant difference among treatments (Figure 5E).

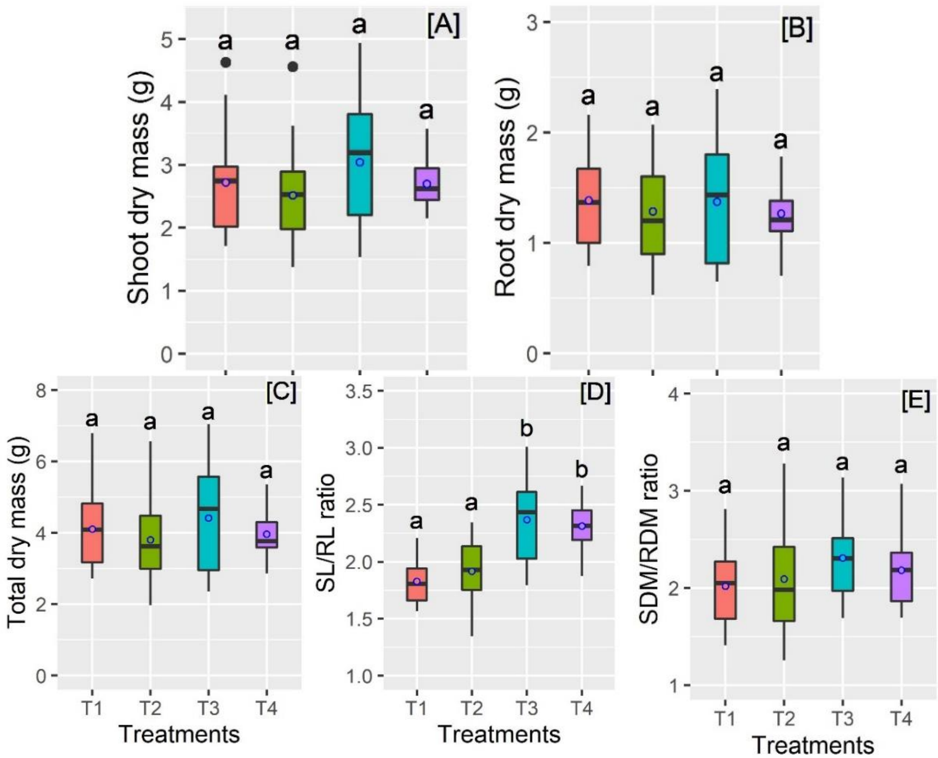

Figure 5. Shoot dry mass (A), root dry mass (B), total dry mass (C), shoot length/root length (SL/RL) ratio (D), and shoot dry mass/root dry mass (SDM/RDM) ratio (E) of *Bertholletia excelsa* seedlings under different sizes after root cut and transplanting. Shoot pruning (T1); small size individuals, ≤ 5 cm in height (T2); medium size individuals, ≤ 11 cm in height (T3); and large size individuals, ≤ 17 cm in height, (T4). The thicker horizontal line represents average, the box the interquartile range and the dashed lines the extreme values. Letters indicate statistical differences (p = 0.05) in ANOVA with Tukey's post-hoc test.

Seedlings quality indexes

Medium and large individuals had highest averages (5.4 ± 0.8 and 5.7 ± 0.7, respectively) of the robustness index, differing significantly from treatments shoot cut (4.2 ± 0.9) and small individuals (4.5 ± 0.6) ($F_{3,76}$ = 17.36, p = 0.001) (Figure 6A). The treatment shoot cut presented highest mean of lignification index (0.43 ± 0.03, $F_{3,76}$ = 11.18, p = 0.001), differing significantly from the other treatments (Figure 6B). In terms of the Dickson Quality Index (DQI), there was a difference between shoot cut (0.66 ± 0.23) and large (0.50 ± 0.09) individuals ($F_{3,76}$ = 2.762, p = 0.047) (Figure 6C).

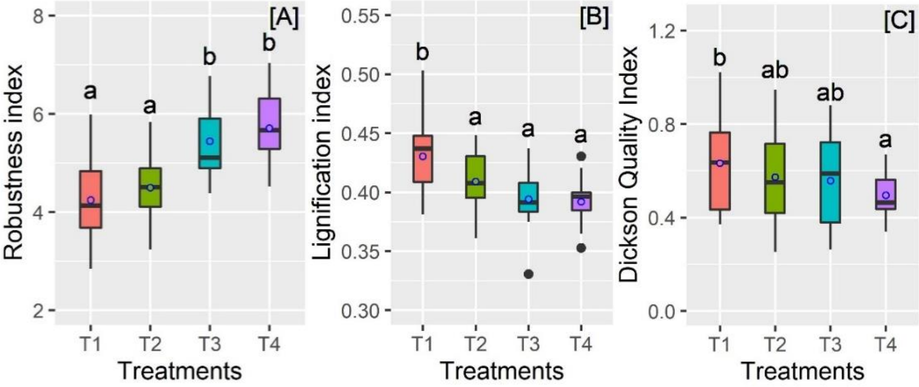

Figure 6. Robustness index (A), lignification index (B) and the Dickson Quality Index (C) of *Bertholletia excelsa* seedlings under different sizes after root cut and transplanting. Shoot pruning (T1); small size individuals, ≤ 5 cm in height (T2); medium size individuals, ≤ 11 cm in height (T3); and large size individuals, ≤ 17 cm in height, (T4). The thicker horizontal line represents average, the box the interquartile range and the dashed lines the extreme values. Letters indicate statistical differences (p = 0.05) in ANOVA with Tukey's post-hoc test.

Table 2. Morphological and qualitative variables of *Bertholletia excelsa* seedlings after transplantation under different sizes and root cut. Shoot pruning (T1); small size individuals, ≤ 5 cm in height (T2); medium size individuals, ≤ 11 cm in height (T3); and large size individuals, ≤ 17 cm in height, (T4).

Variable	**Averages**				**Quality and Interval***		
	T1	T2	T3	T4	Large	Medium	Small
Height (cm)	25.6	25.2	31.3	30.7	15-25	10-14.9	< 10.0
Stem colar diameter (mm)	6.0	5.4	5.5	5.2	≥ 4.0	2.5-3.9	< 2.5
SL/RL ratio	1.8	1.9	2.4	2.3	≤ 2.0	2.1-2.5	> 2.5
SDM/RDM ratio	2.0	2.1	2.4	2.2	1.5-2.0	2.1-2.5	> 2.5
Lignification index	0.43	0.41	0.39	0.39	nd.	nd.	nd.
Robustness index	4.2	4.5	5.4	5.7	≤ 6.0	6.1-8.0	> 8.0
Dickson Quality Index	0.66	0.57	0.56	0.50	≥ 0.5	0.49-0.20	< 0.20

*For conifer species (Reyes *et al.* 2014)
Nd. = Not determined

Optimal intervals were determined to qualify the morphological variables of *B. excelsa* seedlings in forest nursery (Table 2). Regarding the seedling quality, all tested treatments resulted in values equal to or higher than those proposed in the literature. However, the treatment shoot cut attained the highest DQI.

DISCUSSION

Shoot cut (pruning) of the *Bertholletia excelsa* seedlings at the time of the transplanting did not influence the seedlings development and quality, even though the significant effects over some variables such as stem collar diameter and root length (Figure 4 and Table 2). Shoot pruning consists of eliminating part of the seedling's terminal sprout, changing the seedling growth rate. Shoot pruning can favor seedling development, however, it depends on the tolerance level of each species.

Shoot pruning at the time of transplanting the seedlings to the definitive containers can obtain benefits such as: more robust seedlings and an adequate balance of development in shoot and root system. Precocity and uniformity are important characteristics in the seedling production, since the longer the seedlings remain in the forest nursery, higher is the production cost (Auca *et al.* 2018). Production cost is an important variable in seedling production. The need to reduce costs led managers to seek technologies to improve the way how seedlings are produced in nurseries (Ribeiro *et al.* 2018). Environmental factors and silvicultural techniques used are crucial to reduce costs and time to establish a tree plantation (Lima Filho *et al.* 2019).

The SDM/RDM ratio indicates biomass distribution between the shoot and root system (Siqueira *et al.* 2018). Thus, it is important that the container favors the seedling shoot and root growth, so that there is not a big difference between these two parts of the plant. Low SDM/RDM ratio indicates that seedlings have equal distribution of dry matter between shoot and root (Marana *et al.* 2015). The balanced distribution between shoot dry mass and root dry mass allows for adequate plant development, reducing the risk of plant toppling in the field.

Substrate temperature depends on the tube’s volume, varying according to time and space, with an important role in physical processes and energy exchanges with the surrounding environment, which interfere in the shoot and root growth and in the absorption of water and nutrients (Cavalcanti *et al.* 2019). Furthermore, the substrate temperature determines evaporation and aeration, as well as the speed of the chemical reactions involved seedling development (Diniz *et al.* 2014, Nascimento *et al.* 2016). For the production of high quality seedlings it is necessary to use suitable containers, where volume is a crucial variable. Therefore, the container size should be chosen to provide the largest possible volume of substrate to the roots, but with less weight to make the transport to the field easier (Pinho *et al.* 2018).

Seedlings quality indexes

Plant quality is a concept mainly based on morphological and physiological characteristics, which determine its survival and initial growth, depending on local conditions where the individual will be planted. Significant differences were observed for the lignification, robustness, and the Dickson Quality Index (DQI) as a function of seedling size. The robustness index is the relationship between shoot length and root collar diameter, the lower its value, the more robust is the plant. Plants produced in 345-cm³ tubes, for example, have lower robustness index, thus with better quality. This index is an indicator of plant resistance to wind desiccation, survival, and potential growth in dry sites (Reyes *et al.* 2014). The relationship between shoot length and root collar diameter is a non-destructive evaluation method to assess the balance between seedling growth and seedling quality.

The lignification index is related to the lignification of aerial and root tissues of seedlings belonging to woody species under stress. The physical stimulus applied in the form of stem flexion triggers morphometric responses in plants normally associated with a reduction in height, increase in stem diameter and in the dry matter mass of the root tissue (Dranski *et al.* 2015). The response to mechanical stimulus is desirable during the adaptation phase of seedlings to the field. The lignification of seedling tissues has been related to field performance and survival (Dranski *et al.* 2015).

The DQI, as well as the lignification index, are indicators of seedling quality, where the DQI considers vigor and balance of biomass distribution in the seedling (Lima Filho *et al.* 2019). Several authors consider the DQI as the main indicator of seedling quality, as its interpretation considers the robustness and balance of biomass distribution in the seedling, combining growth variables and biometric relationships (Binotto *et al.* 2010, Siqueira *et al.* 2018). Binotto *et al.* (2010) observed that dry mass, followed by stem diameter, are the most strongly variables correlated with the DQI.

Seedling stage is possibly the most vulnerable stage during the plant's life cycle. Under natural conditions, seedling establishment and subsequent reproductive success can be hampered for multiple reasons: premature emergence, germination at improper depths, and emergence in environments with high competitive pressure to survive or leave offspring. Thus, seeds must have effective mechanisms to detect changes in environmental conditions, in order to be able to establish the development of new stages of life (Batlla and Benech-Arnold 2014).

CONCLUSIONS

Shoot cut (pruning) did not present negative effects over the development, growth, and quality of seedlings of the Amazonian tree species Bertholletia excelsa during the first 165 days of seedling production in forest nursery. Seedlings that had the shoot cut presented higher Dickson Quality Index than seedling with no shoot cut, regardless of the initial size of the individuals transplanted. Therefore, shoot cut implies in better performance of B. excelsa seedlings to be planted in the field.

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