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## Effects of pruning and recipient volume on seedlings' quality of *Bertholletia Excelsa* bonpl. (Lecythidaceae)

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### Abstract

*Bertholletia excelsa* is a tree species economically important in Peru, Brazil, and Bolivia. In the Amazon, seedlings production of tree species is a bottleneck faced by many producers. Although the great economic relevance of *B. excelsa*, there are few transplantation methods or techniques that brings more seedlings quality for the species. Thus, the objective of this work was to assess the influence of pruning or shoot cut and the different recipient volumes on the seedlings quality of *B. excelsa*. Thirty days after germination seedlings were transplanted to 115- and 345-cm<sup>3</sup> small plastic tubes filled with standard substrate and arranged in forest nursery under 60% shading. The experiment set up was fully randomized in a 2 x 2 factorial (with and without shoot cut x 115-cm<sup>3</sup> and 345-cm<sup>3</sup> little tubes) in four treatments and four repetitions of 10 seedlings each. ANOVA followed by the Tukey test were applied on

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*the means ( $p < 0.05$ ). Pruning of *B. excelsa* seedlings when transplanted did not influence in the seedlings' quality, even though some variables such as stem collar diameter and root length presented positive responses. *B. excelsa* seedlings produced in 115-cm<sup>3</sup> small tubes presented growth restrictions while seedlings produced in 345-cm<sup>3</sup> small tubes had higher Dickson quality index at 165 days.*

**Keywords:** Brazil nut, tree species, Dickson quality index, Amazon, production in forest nursery.

## INTRODUCTION

*Bertholletia excelsa* Humb. Bonpl. (Lecythidaceae), commonly known as Brazil nut, is a tree species of great economic importance in the Amazon region (ALBUQUERQUE et al., 2015; NOGUEIRA et al., 2018). The extractive activities related to of this species in the Amazon brings employment and incomes for thousands of workers through trade and consume of its seeds (TONINI, 2011; COLES et al., 2016), which present high commercial and nutritional values (BALDONI et al., 2017; WADT et al., 2018). *B. excelsa* is a climax species and light dependent that presents a good growing performance in open areas (ALBUQUERQUE et al., 2015). Trees have large size, with height of 40-60 m and diameter of 1-4 m (SANTOS et al., 2006). Besides this, the species is considered social (MORI; PRANCE, 1990), since it has as an ecological feature a tendency to grow in density clusters of individuals know as Brazil nut lands.

Seedlings production of tree species is one of the most important activities to the establishment of forest plantings, since the seedlings quality has influence on the future plant development. The need of seedling production, for forest restoration or commercial purposes, demands know how, ability, and sensibility of nurserymen in order to acquire techniques that meet quality and low cost. In this case, seedlings must have features that help them to attain maximal survival and rapid initial growth after planting (AUCA et al., 2018; SOUZA et al., 2019). Furthermore, the substrate amount absorbed by the plant can be limited to the recipient volume, which must have an optimal volume to ensure seedling quality at reduced costs (REYES et al., 2014).

Currently there are few recommendations and prescriptions about growing Amazonian native tree species in forest nurseries, which includes the use of alternative recipients and substrates that can promote better growth and quality of *B. excelsa* seedlings. One of the main issues on the seedlings production process of Amazonian native species is that most of them have slow growth, especially those classified late or climax species (FERRAZ; ENGEL, 2011).

Among the factors that influence the seedlings production process of tree species, substrate, substrate humidity, substrate porosity, dormancy, temperature, shading, recipient volume, irrigation, seedlings' quality, fertilization, and the seedlings management in the forest nursery (CAMARGO et al., 2011; COSTA et al., 2015; MARQUES et al., 2018). From these factors, recipients and adequate substrates are crucial to have good seedlings growth and quality under low cost. Although this fact, there is no cultivation system defined for *B. excelsa* (AUCA et al., 2018).

Some cultivation practices can optimize seedlings production, so that pruning immediately after transplanting can promote more robust seedlings and adequate the balance of development in shoot and root length. Regarding recipients, acquisition costs must be considered, as well durability, easy handling, storing, transport, and their availability in local markets. The recipient size must be chosen in the way of promoting the highest possible volume of substrate available for roots, however with lower weight to have easier transport to the field (PINHO et al., 2018). In this way, to associate nutritional power with lower volume recipients is an alternative to reduce costs during the production phase, transport, and seedlings distribution in the field, promoting higher efficiency in yields and planting operations (LIMA FILHO et al., 2019).

Although the great economic relevance of *B. excelsa*, there is no transplantation methods or techniques that brings more seedlings quality for the species. In this context, this work two scientific questions are presented: What are the responses of *B. excelsa* seedlings, in terms of quality, to pruning or shoot cut (a) and to different recipient volumes (b)? The hypotheses raised by the authors were that there are positive responses in quality of *B. excelsa* seedlings to pruning (a) and to the variation of recipient volume (b) Therefore, the objective of this

study was to assess the effects of pruning and recipient volume on the quality of *B. excelsa* seedlings.

## MATERIAL AND METHODS

### Experimental design

The study was carried out in a forest nursery belonging to the Research Institute of the Peruvian Amazon (IIAP), in Puerto Maldonado district, Madre de Dios department, Peru (2°49'11" N, 60°40'24" W). To assess seedlings growth and quality of *Bertholletia excelsa* seedlings, 40 seedlings were transplanted and arranged in four treatments with four repetitions of 10 seedlings each (Table 1).

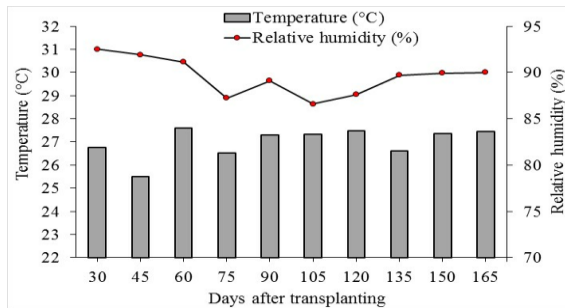
**Table 1.** Treatments used to evaluate initial growth and quality of *Bertholletia excelsa* seedlings during 165 days in forest nursery, Puerto Maldonado, Peru, 2018.

Treatment	#Repetitions/#Seedlings
Shoot cut + 115-cm <sup>3</sup> small tube	4/10
No shoot cut + 115-cm <sup>3</sup> small tube	4/10
Shoot cut + 345-cm <sup>3</sup> small tube	4/10
No shoot cut + 345-cm <sup>3</sup> small tube	4/10

To assess initial growth, the *Bertholletia excelsa* seedlings were selected 30 days after their germination and transplanted to 115- e 345-cm<sup>3</sup> small tubes filled with standard substrate composed by sand, sawdust, and charred sawdust in equal proportions (1:1:1 *v/v*). Seedlings were previously standardized by size and distributed randomly in each treatment (Table 1). Once having seedlings transplanted, the small tubes were arranged in forest nursery covered by a mesh with 60% of shading.

Thirty days after the seedlings transplanting to the forest nursery, the first height measure was done. From the 30<sup>th</sup> day on, measurements were taken a 15-day frequency, totaling 10 evaluations (30, 45, 60, 75, 90, 105, 120, 135, 150, and 165 days). A data logger was used for the daily measurement of temperature and humidity during

the experiment assessment. Eight measures were done per day in intervals of 1 h between them. Means of temperature are presented in 15-day intervals in Figure 1.



**Figure 1.** Mean values of temperature and relative humidity in forest nursery with *Bertholletia excelsa* seedlings in 15-day intervals during 165 days of the experiment evaluation, Puerto Maldonado, Peru, 2018.

### Initial growth and seedlings' quality indexes

The seedlings initial growth was assessed through the variables: a) stem collar diameter, b) shoot length, c) root length, d) shoot dry mass, e) root dry mass, f) total dry mass, g) shoot length/root length relation (SL/RL), h) shoot dry mass/root dry mass relation (SDM/RDM), i) robustness index (RI), j) lignification index (LI), and l) Dickson quality index (DQI). The higher is RI, LI, and DQI, the higher will be the seedlings quality. To measure the stem collar diameter a digital caliper was used (precision = 0.01 mm) and to measure shoot length and root length a ruler graded in millimeters was used.

To evaluate dry mass, seedlings were split in shoot and root through a cut at the stem collar diameter. Both parts were accommodated separately in bags of Kraft paper, identified and dried in an oven at 70 °C during 72 h up to reach a constant mass. Immediately after been removed from the oven, samplings were weighted in an analytical balance (precision = 0.001 g) to obtain shoot and root dry mass.

SL/RL is a relation that foresees planting success, where there must be a balance between the plant's shoot and root systems. It is determined by the following equation:

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

SDM/RDM indicates plant development in the forest nursery, which is given by the equaton:

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

RI is calculated as a ratio between shoot length and the root collar diameter, according to the equation:

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

LI does a relation between the total dry mass with the humid total mass, giving the percentage of lignification. The index is given by the equation:

$$LI = \left( \frac{\text{Total dry mass (g)}}{\text{Total umid mass (g)}} \right) \times 100$$

DQI meets several morphological characteristics in a single value used as a quality index. DQI is determined by the following equation:

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

Optimal intervals were defined to qualify morphological variables of *B. excelsa* seedlings grown in forest nursery. The intervals were divided in low, medium, and high quality, where different measured variables and calculated indexes about *B. excelsa* seedlings produced in forest nursery were compared.

## Data analysis

The experiment set up was fully randomized in a 2 x 2 factorial (with and without shoot cut x 115-cm<sup>3</sup> and 345-cm<sup>3</sup> little tubes) in four treatments and four repetitions of 10 seedlings each (Table 1). To ensure the assumptions of variance analysis (ANOVA), the data were firstly checked regarding to: a) normality with the Shapiro-Wilk test (p > 0.05) and b) homocedasticity by the Bartlett test (p > 0.05). Once met these assumptions, the data were analyzed through ANOVA by the

software R version 3.5.2. In case of significant differences, the means were compared through the Tukey post-hoc test ( $p < 0.05$ ).

## RESULTS

There was no interaction for the variables “small tube volume” and “shoot cut” ( $p = 0.054$ ) (Table 2). Shoot growth in height was significantly higher for seedlings produced in 115-cm<sup>3</sup> small tubes (28.5±3.5 cm,  $F_{1, 76} = 18.146, p = 0.001$ ). Seedlings produced in 345-cm<sup>3</sup> small tubes presented higher growth in stem collar diameter (5.6±0.8 mm,  $F_{1, 76} = 60.579, p = 0.001$ ) and root collar diameter (5.9±1.0 mm,  $F_{1, 76} = 26.346, p = 0.001$ ). Seedlings with shoot cut presented higher stem collar diameter (5.3±0.9 cm,  $F_{1, 76} = 6.983, p = 0.009$ ) (Table 2).

**Table 2:** Mean (±SD) of shoot height, stem collar diameter, root collar diameter of *Bertholletia excelsa* seedlings produced in 115- and 345-cm<sup>3</sup> small tubes.

Variable		Shoot height (cm)	Stem collar diameter (mm)	Root collar diameter (mm)
Small tube volume (cm <sup>3</sup> )	115	28.5±3.5 b	4.7±0.8 a	4.9±1.0 a
	345	25.4±3.8 a	5.6±0.8 b	5.9±1.0 b
Shoot cut	With	26.5±2.8 a	5.3±0.9 b	5.6±1.1 a
	No	27.4±4.2 a	4.9±0.5 a	5.3±0.9 a
CV (%)		11.96	10.86	15.59

Means followed by the same letter in the column do not differ statistically from each other, by the Tukey post-hoc test at 5% of probability.

CV (%) = coefficient of variation.

Under shoot cut, there was interaction between “shoot length” and “root length” of *B. excelsa* seedlings produced in 345-cm<sup>3</sup> small tubes. Seedlings presented higher root growth (14.1±2.5 cm,  $F_{1, 76} = 6.46, p = 0.013$ ) than the other treatments (Table 3).

**Table 3.** Mean (±SD) of the root length (cm) of *Bertholletia excelsa* seedlings produced in 115-cm<sup>3</sup> e 345-cm<sup>3</sup> small tubes.

Small tube volume (cm <sup>3</sup> )	Root length (cm)	
	With	Cut
115	8.7±2.7 aA	8.6±2.8 aA
345	14.1±2.5 bA	13.2±2.5 bB
CV (%)		6.22

Means followed by the same letter do not differ from each other, uppercase in the line and lowercase in the column, by the Tukey post-hoc test at 5% of probability.

CV (%) = coefficient of variation.

A significant difference was detected between treatments for shoot length/root length relation (SL/RL) and shoot dry mass/root dry mass relation (SDM/RDM). Seedlings produced in 115-cm<sup>3</sup> small tubes presented higher shoot dry mass (3.5±0.8 g, F<sub>1, 76</sub> = 26.881), SL/RL (3.3±0.8, F<sub>1, 76</sub> = 312.998), and SDM/RDM (4.5±1.4, F<sub>1, 76</sub> = 204.593) and lower root dry mass (0.8±0.4 g, F<sub>1, 76</sub> = 47.114) than seedlings produced in 345-cm<sup>3</sup> small tubes (*p* = 0.001). Seedlings with no shoot cut had higher SL/RL (2.7±0.9, F<sub>1, 76</sub> = 6.041, *p* = 0.016) than plants with shoot cut (2.5±0.7) (Table 4).

**Tabela 4.** Means (±SD) of the shoot dry mass, root dry mass, relation height/root length (RH/RL), and relation shoot dry mass/root dry mass (SDM/RDM) of *B. excelsa* seedlings produced in 115- e 345-cm<sup>3</sup> little tubes.

Variable		Shoot dry mass (g)	Root dry mass (g)	Total dry mass (g)	RH/RL	SDM/RDM
Small tube volume (cm <sup>3</sup> )	115	3.5±0.8 b	0.8±0.4 a	4.3±0.9 a	3.3±0.8 b	4.5±1.4 b
	345	2.6±0.9 a	1.3±0.4 b	3.9±1.1 a	1.9±0.8 a	2.1±1.4 a
Cut	With	3.1±0.9 a	1.1±0.4 a	4.1±1.0 a	2.5±0.7 a	3.2±1.4 a
	No	3.1±0.9 a	1.1±0.4 a	4.1±1.1 a	2.7±0.9 b	3.3±1.5 a
CV (%)		25.32	31.57	24.59	14.13	22.70

Means followed by the same letter in the column do not differ statistically from each other, by the Tukey post-hoc test at 5% of probability.

CV (%) = coefficient of variation.

Seedlings produced in 115-cm<sup>3</sup> small tubes had higher robustness index (RI) (5.87±1.2, F<sub>1, 76</sub> = 38.881, *p* = 0.001) than plants produced in 345-cm<sup>3</sup> small tubes (4.37±1.2, *p* = 0.001). Differences were also found in the lignification index (LI) (0.42±0.05, F<sub>1, 76</sub> = 49.149) and in the Dickson quality index (DQI) (0.61±0.19, F<sub>1, 76</sub> = 37.818), where seedlings produced in 345-cm<sup>3</sup> small tubes had the highest means (Table 5). There was no influence of the variable “shoot cut” for the RI (*p* = 0.116), LI (*p* = 0.225) and DQI (*p* = 0.222).



**Tabela 5.** Mean ( $\pm$ SD) of the quality indexes of *Bertholletia excelsa* seedlings produced in 115- e 345-cm<sup>3</sup> small tubes.

Variable		Robustness index	Lignification index	Dickson quality index
Small tube volume (cm <sup>3</sup> )	115	5.87 $\pm$ 1.2 b	0.36 $\pm$ 0.05 a	0.41 $\pm$ 0.16 a
	345	4.37 $\pm$ 1.2 a	0.42 $\pm$ 0.05 b	0.61 $\pm$ 0.19 b
Cut	With	4.95 $\pm$ 1.2 a	0.49 $\pm$ 0.05 a	0.53 $\pm$ 0.19 a
	No	5.29 $\pm$ 1.2 a	0.39 $\pm$ 0.05 a	0.49 $\pm$ 0.16 a
CV (%)		18.65	10.35	28.24

Means followed by the same letter in the column do not differ statistically from each other, by the Tukey post-hoc test at 5% of probability.

CV (%) = coefficient of variation.

The optimal intervals defined to qualify the morphological variables of *B. excelsa* seedlings in forest nursery are presented in Table 6. In relation the seedlings quality, those produced in 345-cm<sup>3</sup> small tubes reached the best values.

**Table 6.** Mean values of morphological characteristics to evaluate quality of *Bertholletia excelsa* seedlings.

Variável	Qualidade e intervalo		
	Alta	Média	Baixa
Shoot length (cm)	25.0-35.0	15.0-24.9	< 15.0
Stem collar diameter (mm)	$\geq$ 5.0	3.5-4.9	< 3.5
Relation shoot length/root length	$\leq$ 2.0	2.1-2.5	> 2.5
Relation shoot dry mass/root dry mass	1.5-2.0	2.1-2.5	> 2.5
Lignification index	$\geq$ 0.5	0.35-0.49	< 0.35
Robustness index	$\leq$ 5.0	5.1-7.0	> 7.0
Dickson quality index	$\geq$ 0.5	0.49-0.30	< 0.30

## DISCUSSION

Pruning, or shoot cut, of *Bertholletia excelsa* seedlings when transplanted did not influence seedlings' quality, even though some variables such as stem collar diameter and root length had responded to pruning (Tables 2 and 3, respectively). This result showed the rejection of out hypothesis. Shoot pruning consists in in eliminating part of the seedlings terminal bud, altering the seedlings growth rate, which can be good for the seedling development. However, pruning depends on the tolerance level of each tree species.

Pruning at the moment of seedlings transplanting to the recipients can benefit as more robustness, and good development

balance between shoot and root. Precocity and uniformity are important features for seedlings production, once the more time seedlings remain in the forest nursery the higher will be production cost. Financial cost is a relevant variable for seedlings production, which drives foresters to look for new technologies to improve the ways how to produce seedlings in forest nurseries (RIBEIRO et al., 2018). Besides this, environmental factors and silvicultural techniques employed are crucial to reduce cost and time to establish a tree planting (LIMA FILHO et al., 2019).

The root growth of *B. excelsa* seedlings had effects of the recipient size. Plants produced in 345 cm<sup>3</sup> small tubes presented higher development of their root system. This variable influences on the shoot length/root length relation (SL/RL) and on the shoot dry mass/root dry mass relation (SDM/RDM) (Table 4). SDM/RDM indicates the biomass distribution between shoot and root systems (SIQUEIRA et al., 2018). Thus, it is important that the recipient favors both the shoot and root growth in order to avoid unbalance in growth between these two plant's parts. Low values of SDM/RDM indicate that seedlings have similar distribution patterns of dry matter among organs (MARANA et al., 2015). The balanced distribution between shoot dry mass and root dry mass makes possible an adequate seedling development, decreasing risks of plant fall in the field. Such values showed better SDM/RDM of seedlings produced in 345-cm<sup>3</sup> small tubes. The substrate temperature is influenced by the small tube volume, which is a variable space and time dependent. It has an essential role in the physical processes and in the energy exchanges with atmosphere, so this interferes on the growth of buds and roots and in the water and nutrients absorption (CAVALCANTI et al., 2019). Moreover, the substrate temperature determines evaporation and earation, as well as the speed of chemical reactions involved in the seedling development (NASCIMENTO et al., 2016). For the production of quality seedlings it is necessary the use of proper recipients, which includes volume the volume of substrate available for the seedling. The recipient size must be chosen taking into account a balance between the highest possible volume of substrate for roots and the lowest weight to make seedlings transport tot the field easier (PINHO et al., 2018).

### **Seedlings' quality indexes**

The concept of seedlings' quality is based mainly on the morphological fisiological features of the individual that determine survivorship and initial growth in function of the environment where the seedling will be eventually planted. Regarding the small tube volume, significant differences were found for the lignification index (LI), and for the robustness index (RI) and DQI. RI is a relation between seedling's height and the root collar diameter, so the lower its value more robust is the plant. Seedlings produced in 345-cm<sup>3</sup> small tubes presented lower RI and consequently better quality. This finding comes to corroborate the initial authors' hypothesis. RI is a non-destructive evaluation method that considers the balance between the plant's growth and its quality. Hence, this is an indicator of the seedling resistance to desiccation by Wind and potential growth in dry sites (REYES et al., 2014).

Seedlings produced in 345-cm<sup>3</sup> small tubes presented higher LI. This index is the ratio between total dry mass and total humid mass that results in a percentage of lignification. Moreover, LI is related to the lignification of the aerial and root tissues of wood species seedlings under stress. As much higher is LI as much higher are lignified the plant tissues. The physical stimulus applied as stem flexions triggers morphometric responses in the plants normally associated to a higher lignification, height reduction increase in stem collar diameter, and in the root dry mass. The response to the mechanical stimulus is called thigmomorphogenesis. During the seedlings rustification phase, the thigmomorphogenesis is desirable, which gives higher survivorship chances in the field (DRANSKI et al., 2015).

DQI, as well as LI and RI, is considered as an indicator of seedlings quality. In this index are taken into account the seedlings vigor and the distribution balance of its biomass (LIMA FILHO et al., 2019). DQI is the main indicator of the quality standards of seedlings, since in its composition there are robustness and balance of biomass distribution in the seedling, meeting variables of growth and biometric relations (DICKSON, 1960; SIQUEIRA et al., 2018).

The seedling stage is the most vulnerable time of a plant's life cycle. In natural conditions, the seedling establishment and further reproductive success stopped are threatened due to several factors such as premature germination, seeds in so deep places, germination in sites

under high competitive pressure for survivorship and reproduction. Thus, seeds must have efficient mechanisms to detect changes in the environmental conditions in order to attain new developmental stages (BATLLA and BENECH-ARNOLD, 2014).

## RECOMMENDATIONS

The seedlings quality was not affected by shoot cut (pruning). On the other hand, the recipient volume had effects on the seedlings quality. Hence, we recommend the use of 345-cm<sup>3</sup> small tubes for the *B. excelsa* seedlings production. The use of little tubes for the production of *B. excelsa* seedlings has not been reported in literature. However, we recommend the use of this kind of recipient, since the 345-cm<sup>3</sup> small tube did not cause roots folding and promoted good seedlings development.

An important feature of *B. excelsa* seedlings is their rusticity once managed in forest nursery. Even presenting high rusticity, it is essential a minimum infrastructure in conditions to protect seedlings against attacks of rodents and ants. The use of small tubes for seedlings production in forest nurseries can improve several steps of the process as cheaper transport of seedlings to the field and easier handling and better ergonomic conditions for forest workers.

Regarding the seedlings quality for the field (Table 6), we suggest intervals that, if correctly adopted, can ensure *B. excelsa* seedlings with quality and good development in the field. Based on values of the last measurement (165 days), only seedlings produced in 345-cm<sup>3</sup> small tubes presented the ideal characteristics for the field (Table 6). One of the main issues found in forest nurseries of native tree species is the fact that seedlings must remain there up to reach the ideal conditions for planting. This may provoke problems for the root system, as a reduction in its physiological activity, deformations, and root necrosis, due to the lack of space and nutrients in smaller recipients (LOPES et al., 2014). Therefore, based on the data of the last measurement on height and stem collar diameter and on the indexes SDM/RDM and DQI (Table 2), *B. excelsa* seedlings produced in 345-cm<sup>3</sup> small tubes were significantly better than seedlings produced in 115-cm<sup>3</sup> small tubes. In this way, this technology becomes more recommended for production of *B. excelsa* seedlings, when the objective

is to obtain seedlings with higher quality in shorter time for planting in the field.

## CONCLUSIONS

*Bertholletia excelsa* seedlings had no effects of pruning or shoot cut on their development and quality.

*Bertholletia excelsa* seedlings produced in 115-cm<sup>3</sup> small tubes had restrictions on root growth, which resulted in lower shoot length/root length relation and shoot dry mass/root dry mass relation.

*Bertholletia excelsa* seedlings produced in 345-cm<sup>3</sup> small tubes presented higher Dickson quality index

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