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Vegetative Propagation of Amazonian Indigenous Species for Restoration Practices Over a Riverscape Floodplain Disturbed by Silting

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Abstract

The use of native species' stem cuttings in riparian forests disturbed by silting could be a promising low-tech alternative for restoration practitioners in riverscape rehabilitation. In this study, we evaluated the vegetative propagation of Amazonian native plants (Buchenavia parviflora, Euterpe oleracea, Ficus insipida and Socratea exorrhiza) with the addition of a bio-fertilizer, and humic and fulvic acids in soil disturbed by human-induced silting. We found that F. insipida and B. parviflora were able to grow and showed high survival percentage with the development of leaves, buds, and roots; even in nutrient deficient and clayey soils. We also found that the frequency of application and the concentration of the organic additives did not show significant influence on plants' development. Thus, advance in situ tests with both species could be an interesting step to contribute to riverine ecosystems restoration practices.

Keywords: Cuttings, biofertilizer, low-tech, aggradation, riparian forest.

1. INTRODUCTION AND OBJECTIVES

Riverscape aggradation by heavy loads of thin particles due to land use is an enormous challenge that riverine ecosystems are facing worldwide. This challenge is specially hard in areas of the Amazon forest rich in mineral resources (Bryant & Büscher, 2015; Lobo et al., 2018).

Vegetation can be cost-effective and crucial to river morphodynamics in the role of physical ecosystem engineers for river restoration (Beechie et al., 2010; Brierley & Fryirs, 2004; Roni & Beechie, 2013). However, recovering techniques for riparian forests using seed propagation may not be suitable in every scenario due to wider deposits and compact soil conditions found in some disturbed areas.

The use of cuttings of native species for vegetative propagation could be a promising low-tech alternative for restoration practitioners in riverscape rehabilitation since this technique could overcome the thin particles layer and be adapted to specific local conditions (Dias et al., 2012; EMBRAPA, 2002; Ferrari, Grossi & Wendling, 2004; Oliveira et al., 2001). Furthermore, the cuttings can reduce the impact of rain and erosion on the soil, decrease the runoff velocity and magnitude and increase water infiltration and soil cohesion (Durlo & Sutili, 2012).

There are a lot of studies regarding cuttings and the use of growth regulators; as Auxins, Cytokinins, Ethylene, and other potential hormones (Cézar et al., 2009; Endres et al., 2007; Ferreira et al., 2010; Leandro & Yuyama, 2008; Pacheco & Terezinha, 2008; Sampaio et al., 2010; Santos et al., 2011; Souza et al., 2009; Tiberti et al., 2012; Voltoni, Girard & Aparecida, 2007). But only few studies consider the natural response of plants to grow without the addition of hormones stimulators (Davies et al., 2017; Ferriani et al., 2011; Kibbler, Johnsto & Williams, 2004; Nicoloso, Lazzari & Fortunato, 1999; Wendling, Ferrari & Ferreira Dutra, 2005). Although the non-use of growth regulators makes the method easier to perform, fast regeneration of roots and leaves in the cuttings is necessary for the reestablishment of the plant's water homeostasis, transportation of nutrients and the photosynthesis process (Davies et al., 2017).

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Bio-fertilizers are biologically active compounds resulting from the fermentation of organic matter by microorganisms; they are rich in enzymes, antibiotics, vitamins, hormones and have soil inoculum microorganisms (Busato et al., 2016; Fontenelle et al., 2017). Bio-fertilizers can be produced by local communities and could be an important ally to avoid the use of commercial high cost additives. The application of bio-fertilizers can be benefited by traditional ethnopedology, as the Mebêngôkre knowledge to apply organic matter and forest detritus inside small holes in the forest (Museu Paraense Emílio Goeldi, 1987). Also, test responses of different tree species to low-technology propagation methods, as the cuttings method, could generate knowledge to be transferred to local populations and restoration practitioners (Danthu et al., 2002; Leakey, 1990).

Humic and fulvic acids, usually found as the product of soil litter decomposition in the Amazon forest, can additionally increase the plants' growth response due to their interactions with soil microorganisms and compounds (Boveiri Dehsheikh et al., 2020; Gomes Júnior et al., 2019; McClain et al., 1997). They can act as a rich energy resource for soil microorganisms, increasing their numbers and activity and boost the bioavailability of nutrients in the soil (Leite, Iwata & Araújo, 2014).

Besides the usage of common hardwood plants, palm trees can be an interesting choice for alternative methods to recover disturbed riparian forests as there are many dominant groups adapted to riparian forest and wet soils.

Therefore, the aim of this study was to evaluate the vegetative propagation of Amazonian native plants by cuttings and the use of biofertilizer and organic acids to stimulate their development in disturbed clayey soils found in man-induced aggradation areas in Amazonian riverscapes.

2. MATERIALS AND METHODS

We made the experiment in the Native Plant Nursery Complex in Carajás National Forest, in the State of Pará, Brazil, from February to April 2018 in a humidity and temperaturecontrolled greenhouse. The Complex also provided each stock plant used in this experiment.

We selected four native species which are largely found in watershed lower and wet areas: *Buchenavia parviflora* Ducke (*B. parviflora*), *Euterpe oleracea* Mart. (*E. oleracea*), *Ficus insipida* Wild. (*F. insipida*) and *Socratea exorrhiza* (Mart.) H. Wendl. (*S. exorrhiza*).

We sectioned the cuttings from the base of their stock plants, with approximately 20 cm of length and 0.5 cm of width, in a basal shape cut as a bevel (Pio et al., 2003; Sampaio et al., 2010; Santos et al., 2011). Then, we put the cuttings immersed in trays with water for humidity maintenance until experiment deployment on soil (Davies et al., 2017).

To increase plants' growth rate and development success, we provided mineral nutrients using two additives besides the soil components: a modified bio-fertilizer developed by Embrapa Hortaliças (BF); a commercial humic and fulvic acids product (HUM-I-SOLVE) (HA); and a black soil composed by plant litter, black soil, sand and NPK 5% (Busato et al., 2016; Canellas & Olivares, 2014; Fontenelle et al., 2017).

We prepared the bio-fertilizer mixture with rice flour, castor meal, bones meal, birds manure, crushed seeds, brown sugar, cassava and water, all fermented by efficient microorganisms (Busato et al., 2016; Fontenelle et al., 2017). Due to the unavailability of two components (ashes and blood meal), we modified the original recipe and used bird manure instead. The fermentation occurred for seven days, then the hold time and microorganism's adaptation took additional seven days before the first use in fertigation. We diluted the mixture to 2%.

The following specifications were obtained from the HA technical report: Boron (B) = 1%; Total Organic Carbon = 4.5%; Saline Index = 4; Density = 1.05; pH = 7.8. We applied the fertigation with BF/HA once a week starting 10 days after the beginning of the experiment.

We tested three substrates to investigate plants' development potential in areas disturbed by heavy loads of thin particles: i) One mixed organic substrate composed by 30% black soil, 35% forest litter and 35% clay (control); ii) One bare soil composed by silt and clay particles sampled from a disturbed area (clay); iii) One prepared by a vertical substrate profile (clay-mix), (a first upper layer of 0-10cm composed by substrate ii (clay) and a second layer of 10-30cm composed by substrate i (control) (Figure 1). The third substrate treatment was prepared as we think could be a possible planting field intervention scenario, where the cutting would have to overcome the clayey layer, adjusted to the length of the cuttings used in this experiment.

We prepared the experiment by organizing boxes following a 4 x 3 x 2 randomized design. That means 4 plant species, 3 substrates and 2 types of treatments with additives (with and without BF/HA); and 5 replicates. The cuttings were planted leaving 10cm below the substrate's surface and 10cm above. We followed the experiment for 45 days to get a reasonable response of a number of leaves, buds and rooting development (Leandro & Yuyama, 2008; Oliveira et al., 2008; Santos et al., 2011; Tiberti et al., 2012). We applied the water irrigation 3 times every week during the 45 days.



Figure 1. Experiment design with its' substratum treatments (clay; clay-mix; and control) in three humidity sectors with different distances from the wet wall (sector 1 = 5-6m; sector 2 = 3-4m; sector 3 = 1-2m) during the experiment inside the greenhouse at the Native Plant Nursery, in Carajás National Forest.

To evaluate the characteristics of the substrate, we sampled 3 replicates of each treatment on the first and last days of the experiment. We measured concentrations of bioavailable phosphorus (bioavailable P) and total phosphorus (total-P) using the colorimetric method of the molybdic acid measured in a Beckman spectrophotometer (APHA, 1998). Total carbon (total-C) concentrations were estimated by TOC-5000 Carbon Analyzer (Shimadzu) through the oxidation of organic carbon at high temperature and CO reading, formed with a platinum catalyst (Fonseca et al., 2006). To evaluate differences among treatments (substrate types and BF/HA presence and absence) individually, for the nutrients cited above we performed a Kruskal-Wallis analysis ($p \le 0.05$).

To evaluate moisture content among substratum types and humidity sectors inside the greenhouse, we did a twoway ANOVA ($p \le 0.05$). The humidity sectors were based in the distance from the experiment to the wet wall used to maintain humidity inside the greenhouse (sector 1 = 5-6m; sector 2 = 3-4m; sector 3 = 1-2m). To help us understand the differences in the response variables (leaves, roots, plant length, and stem diameter) between the stock plant species selected after survival evaluation (*B. parviflora* and *F. insipida*) we tested data by Mann Whitney U Test ($p \le 0.05$); For differences between cuttings from both species (*B. parviflora* and *F. insipida*) regarding leaves and roots, we tested data by t-test ($p \le 0.05$). To evaluate differences between the number of leaves, buds, and the length of the roots among *B. parviflora* and *F. insipida* cuttings' considering each type of treatment (substrate type and presence/absence of BF/HA), we did a two-way ANOVA ($p \le 0.05$). Roots' length data were log transformed to fit parametric requirements. All statistical analyses were performed using Past v3.25 and GraphPad Prism v8.

3. RESULTS

Plant species was a key characteristic distinguishing plant development between treatments in this experiment. Substratum treatment did not show a major effect on the variability of samples. Nevertheless, between substratum and nutrient addition, the former remained the primary factor for plant development considering the low variability found among treatments of the same plant species.

B. parviflora developed a higher number of leaves than *F. insipida* regardless of the substratum condition (clay, mix, and control) and presence/absence of nutrients (BF/HA). However, the species *F. insipida* showed a quick development of its roots extension, higher than *B. parviflora*, contrasting with the initial differences found on their stock plants, where *B. parviflora* showed more leaves and longer root system on the same stage of development as *F. insipida*.

3.1. Substratum moisture content

Regarding the humidity gradients inside the greenhouse and the distance among replicates and the wet wall, we did not find a high variability between the moisture content of each substratum during the experiment (Figure 2). Data suggested a significant difference in substratum type (2-Way ANOVA F = 10,02; p < 0.05) (Figure 2), but we did not find any direct influence for the development of leaves, roots, and buds of those replicates.



Figure 2. Box-plot (median and Tukey) showing the soil moisture content (%) differences (F = 5.33; p < 0.05) among substratum treatments (clay; clay-mix; and control) in three humidity sectors based in the distance from the experiment to the wet wall (sector 1 = 5-6m; sector 2 = 3-4m; sector 3 = 1-2m) during the experiment inside the greenhouse at the Native Plant Nursery, in Carajás National Forest.

3.2. Substratum nutrients

Results showed that after introducing BF/HA, the substratum of each treatment showed a slightly higher variability of total-P and total-C, and a slightly lower concentration of bioavailable P (Figure 3).

We did not submit any substrates to BF/HA before the beginning of the experiment. Therefore, the concentration of each factor (bioavailable P, total-P, and total-C) was different among each substrate. As expected, the bioavailable P and total-C showed lower concentration in the clayey substratum than in the other treatments (Figure 3).

3.3. Survival

In order to consider that the treatments were successful after 45 days, we should be able to find leaves and roots development. Therefore, we tested which species developed those features in control soil and showed over 50% of leaves and roots development per individual (at least one leaf and short root development) (Figure 4). Our results showed that only *B. parviflora* and *F. insipida* developed a significant number of individual leaves and roots on the proposed experiment, we selected both for posterior tests.



Figure 3. Box-plot (median and Tukey) showing the concentration of Bioavailable Phosphorus (P) (mg/g) on graph A (H = 17.52; p < 0,05); Total Phosphorus (Total-P) (mg/g) on graph B (H = 13.76; p < 0,05); Total Carbon (Total-C) (mg/g) on graph C (H = 16.21; p < 0,05); among substratum treatments (clay; clay-mix; and control) submitted or not to bio-fertilizer and humic and fulvic acids addition after 45 days of substratum exposure to plants and microorganisms metabolism.





Figure 4. Presence of leaves (A) and roots (B) among plants individuals (%) for each species tested for vegetative propagation (*B. parviflora*; F. insipida; *E. oleracea* and *S. exorrhiza*).

3.4. Stock plants

In the number of leaves, the species' stock plants were significantly different. *B. parviflora* had an average almost 8 times higher than *F. insipida*, and *B. parviflora* varied it's number of leaves more than *F. inspida* (U = 0.5; p < 0.05; n = 30) (Figure 5). Regarding the data on the length of the root, the species were also different. *B. parviflora* showed roots two times longer than *F. insipida*, but in contrast to what was shown in leaves, the numbers for the former varied less than the ones for the later (U = 72.5; p < 0.05; n = 30). *F. insipida* plant length was longer than the *B. parviflora* (U = 26.5; p < 0.05; n = 30), but the diameter of the stem was similar for both species (U = 401.5; p = 0.4; n = 30).

Between the cuttings species that developed leaves; *B. parviflora* averaged 5.76 (±3.98) leaves per plant cutting and was significantly higher (U = 294.5; p < 0.05) than *F. insipida*, which averaged 3.53 (±2.24) (Figure 6). For root length, *F. insipida* averaged 9.26 (±7.26) cm and was significantly longer (U = 176; p < 0.05; n = 30) than *B. parviflora* 2.73 (±2.86) cm (Figure 6).

3.5. Plants responses to substratum and biofertilizer addition

Our second approach was to investigate if the species which showed vegetative propagation could develop in bare clay soil and layered clay/mixed soil conditions in the presence or absence of BF/HA; and compare to the development in mixed organic soil (control).

Among all response variables for both species, the greatest effect of variation was found within substrates and no statistical differences were found among treatments (Figure 7 A-E). There was the exception of the number of buds for *F. insipida* that showed a higher percentage of variation in the presence/absence of BF/HA (18.56%), and less than 1% of variation was found among substratum treatments. Thus, treatments considering the type of substrate were consolidated. We found significant differences between the treatments BF/HA (U = 57; p = 0.02; n = 12); in the presence of BF/HA we found a greater number of buds (Figure 7–F).



Figure 5. Violin-plot (Interquartile and median) showing the number of leaves (units) (U = 0.5; p < 0.05) (Graph A); root length (cm) (U = 72.5; p < 0.05) (Graph B); plant length (cm) (U = 26.5; p < 0.05) (Graph C); and stem diameter (cm) (U = 401.5; p = 0.4) (Graph D) of *B. parviflora* and *F. insipida* stock plants. Violin plots provide summary statistics typical of boxplot accompanied with a visualization of the probability density of the data.



Figure 6. Violin-plot (Interquartile and median) showing the number of leaves (t = 2.67; p < 0.05) (A), and root length (cm) (t = 3.22; p < 0.05) (B) for both species *B. parviflora* and *F. insipida*, which succeeded in vegetative propagation during the present experiment. Violin plots provide summary statistics typical of boxplot accompanied with a visualization of the probability density of the data.



Figure 7. Violin-plot (Interquartile and median) showing the number of leaves of *B. parviflora* (F = 1.46; p = 0.25) (Graph A), and *F. insipida* (F = 0.77; p = 0.47) (Graph B); the root length of *B. parviflora* (F = 1.30; p = 0.29) (Graph C), and *F. insipida* (F = 1.91; p = 0.17) (Graph D); the number of buds of *B. parviflora* (F = 2.23; p = 0.12) (Graph E), and *F. insipida* (F = 0.11; p = 0.89). Violin plots provide summary statistics typical of boxplot accompanied with a visualization of the probability density of the data.

4. DISCUSSION

B. parviflora and *F. insipida* developed leaves, roots and buds after 45 days of experimentation, even in poor substratum conditions (clay treatment). However, each species showed its own particular advantage for settlement; this is probably due to the differences in the natural plant development between the two species in regards to their evolution, history and features (Machado et al., 2018; Stace, 2007).

Contrary to what we expected, the substratum type and BF/ HA addition did not show major effects on the development of plants, except for the number of buds for *F. insipida*. The lack of nutrient needs in the initial stage of the cutting's development was probably the reason why the plants did not split into groups divided by P availability, even with the substantial differences found in the concentration of substratum nutrients.

The average soil moisture content found during this experiment was between the wilting point (driest) and field capacity (wettest) (20-40%, respectively), which is usually found in clayey soils (Brandt et al., 2017). This observation was important to certify that the substratum moisture was not low enough to "disturb" hydric conditions for plant development or high enough to lead to leaching on waterlogged soils or to contribute to rot by other microorganisms. Notwithstanding, this characteristic did not contribute to suitable wet conditions for the development of the palm trees used in this experiment; which usually grow in waterlogged soils (Goldstein & Santiago, 2016; Ulrich Lüttge, 2008). Data on total-P highlighted the soil capacity to hold P after BF/HA additions, especially for clayey and clay-mix substratum. In contrast, the concentration of bioavailable P was slightly lower for both organic soils (clay-mix and control) after the experiment, showing that the processes of P sorption were probably decreased and mineralization was favored with the inoculation of others organic compounds and microorganisms consequently, microorganisms and plants uptake were higher (Barker & Pilbeam, 2007).

The results showed lack of nutrients available in clayey soils. There was, however, presence of organic matter. Its origins were probably the small detritus of grass found on the subsurface layer of the soil sampled from the slowly paced natural regeneration of the disturbed area.

Although the substratum moisture was enough for the development of the species *B. parviflora* and *F. insipida*, *E. oleracea* and *S. exorrhiza* are hydrophytic species with pneumatophores which is essential for root respiration in wetlands areas, one of the reasons of its widespread predominance in the Amazonian swamps and floodplains (Avalos et al., 2005; De Oliveira & Schwartz, 2018; Goldsmith, Gregory & Zahawi, 2007; Schauss, 2016). Thus, the moisture content used in this experiment was not enough to provide an ideal condition for the development of palm trees.

In the juvenile phase (10 months past seeds dormancy break) the differentiation of stipes and leaves is still not clear, but we made the cuts of each plant trying to preserve the unique apical meristem in the crown's top, so the plant would be able to generate new organs (Cohen, 2017). Therefore just a few stipes of *E. oleraceae* and *S. exorrhiza* could generate adventitious roots.

Although we used juvenile plants (chronological age), we did not certify their ontogenetic age and the relationship between vegetative propagation capacity during a specific phase of their development. Also, due to the natural lack of meristems, the ramification of the main stem is rare, and any minor damage in the shoot apical meristem can kill the plant (Avalos et al., 2005). Therefore, the process of cutting can be hard for those species, and this can turn the vegetative propagation of the Arecaceae family hard to achieve.

We tried to conduct the experiment using those species due to their widespread occurrence in the study area. Initially, this seemed to be great to ensure the adaptations to the local climate and other environmental pressures which those plants could find after the success of the method selected (cutting) for restoration practices, as the strategy to overcome the clay layer deposited in the area of interest. Instead, we found some challenges to develop those plants by vegetative propagation.

In our experiment, *F. insipida and B. parviflora* responded to vegetative propagation, and their survival chance was high

considering the development of leaves, buds, and roots (>70% and >95% for *B. parviflora* and *F. insipida*, respectively).

As *B. parviflora* and *F. insipida* showed important survival feedback to vegetative propagation, and as they show a natural occurrence in the Amazon forest, especially the riparian forest area, they may be important alternatives for restoration programs considering the cutting technique within this biome (Schöngart et al., 2007).

Although we have found some studies related to Combretaceae and Moraceae families and Ficus genus researches, no application was found of the cutting technique for the species used in this experiment (Danthu et al., 2002; Leakey, 1990; Sarma, 2002). For other species, in a timeframe between 25 and 180 days: Kettenhubber et al. (2019) found survival percentage with roots formation in two different seasons between 5 and 100% for seven riparian forest species found in the Atlantic forest biome in Brazil; Bispo et al. (2016) found survival percentages with roots formation of 69.5%, 75.5% and 91.5% for medial cuttings of Lippia insignis (Moldenke), Lippia lasiocalycina (Cham), and Lippia thymoides (Martius & Schauer), respectively; Matsumoto et al. (2009) found 72% survivability with roots formation for Dioscoreaalata; Sampaio et al. (2010) found 64% survivability with buds and roots formation for Aniba canelilla (H.B.K. Mez); Betanin et al. (2010) found 35.4% survival success with roots formation for Erythrina falcata (Benth.); Aiello et al. (1998), found 4% and 35% survival rate with roots formation for Amelanchier lamarckii and Prunus serrulate (Kwanzan), respectively; Leandro et al. (2008) found 25% survivability for *Couepia edulis* with roots formation.

The characteristics of the stock plants showed positive correlations with their derived cuttings' main characteristics (leaves and roots). *B. parviflora*, which showed a higher number of leaves in its stock plants, also showed a slightly higher number of leaves in its cuttings. *F. insipida*, which showed longer roots within its stock plants, also exhibited longer roots in its cuttings. This result seems to be an important cutting indicator of plant tissue development and could guide restorations practitioners to select species for experiments before field deployment.

Although there were different approaches and treatments regarding substratum type and BF/HA addition, we did not find significant differences considering the number of leaves and root length in most cases within the same species. We believe that these findings could have been caused by a short period experiment after cuttings deployment and the low frequency of bio-fertilizer fertigation.

Despite the fact that 45 days are below the average that we found in the literature for experiments monitoring the cutting technique without hormone stimulators (Davies et al., 2017; Ferriani et al., 2011; Kibbler, Johnston & Williams, 2004; Nicoloso, Lazzari & Fortunato, 1999; Wendling, Ferrari & Ferreira Dutra, 2005), the timeframe was enough to find more than slight vestiges to interpret results for our response variables (leaves, roots, and buds development).

Along with the bio-fertilizer preparation, we modified the timeframe for fermentation and some components to attend the time available to conduct the experiment in the greenhouse and the items available in the local plant market. The mixture fermented for 7 days and the first inoculation happened in another 7 days after that; when the microorganisms' density is close to the optimum (10 days), as recommended by other studies (Busato et al., 2016; Fontenelle et al., 2017; Souza et al., 2012).

As the key components were used, we do not believe that those modifications could have changed the bio-fertilizer's effect. We also used the dilution recommended by previous studies for seedlings development (2%) to provide enough nutrients and to avoid electrical conductivity issues (Busato et al., 2016; Fontenelle et al., 2017; Souza et al., 2012).

However, as the timeframe used by the Busato et al. (2016) experiment was 60 days, and the plant development differences between control and biofertilizer treatments were higher in the Fontenelle et al. (2017) experiment using a constant irrigation process provided by the drip technique; we believe that 45 days and the fertigation frequency (once a week) may not have been enough to uncover the potential of the HORTBIO bio-fertilizer.

5. CONCLUSIONS

Although the quick development of leaves appears as an important feature to describe success after the cuttings deployment, the root system structure is a significant feature to maintain the plant fixed in the soil for applications over stream banks and riparian forests. Those areas are commonly submitted to high runoff and erosion during flood dynamics in the streams water cycle.

We believe that this result suggests that the species selected for the next phase of cuttings tests for the restauration of this kind of environment should be able to prolong their roots to reach a deeper soil horizon. Hence, overcoming the surface clayey deposits, increasing anchorage potential and accessing more nutrients to plant development. The survivability rate is also fundamental to increase the chance of settlement in hard conditions. As the initial application purposes of this experiment was to find plants species to enhance recovering strategies in river banks and riparian forest both disturbed by higher aggradation of clayey material, *B. parviflora* and *F. insipida* showed prominent results, but *F. insipida* showed an advantage to be selected for posterior field experiments with its faster roots' development and its massive survival percentage with roots development of 95%.

As the next phases of the research seek for an alternative method to grow plants with low-tech restoration applications, we choose to check the plant development in disturbed soil conditions with the addition of a bio-fertilizer produced by regional forest microorganisms and common local plant market items instead of using commercial hormones. In this context, we are optimistic about what we can achieve in the next phase using those species in bare or poor nutrients soils.

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