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Base saturation in mixed substrate for seedlings of *Eugenia uniflora* and *Eugenia involucrata*

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Abstract
Given the occurrence of a vast amount of degraded areas in Brazil, recovery projects have been necessary. In order to propose a feasible project, we must be concerned about the production of seedlings, preferably native ones, using low-cost inputs. In this research, we aimed to evaluate the seedlings growth of two forest species (*Eugenia uniflora* and *E. involucrate*) under the influence of different base saturation of a substrate. To this, we tested different dosages of dolomitic lime applied to a substrate composed of soil and sand in a 70/30 ratio. By varying the base saturation of substrate at 40%, 45%, 50%, 55% and 60%, five treatments were tested in a completely randomized design with five repetitions of three seedlings per treatment. We verified that in a mixed substrate of soil and sand the base saturation equal to 45% was the best one to improve the height growth of *E. uniflora* and *E. involucrata* seedlings during one year of evaluation. There was no significant difference among treatments in relation to the variable collar diameter and height/collar diameter ratio for *E. involucrata*.
Keywords: Native species, Forest nursery, Silviculture

Introduction

The expansion of cities and crop areas brought disastrous consequences to native ecosystems conservation. It has been happening by means of different forms of degradation that create degraded areas with different resiliencies, what also influence the quality of life of people living in urban and rural areas, due to water, soil and air contamination (Foley et al. 2005).

When an area still shows its stability and resilience after a degradation process, one of the simple techniques of recovering is the planting of native forest species by means of different strategies to cover the soil and mix species (Durigan et al. 2010). For this, the concern about species diversity is a relevant task because this can make possible a greater interaction of trees and native birds and other microorganisms, with the aim of ensuring the sustainability of restored forests (Andrade et al. 2007; Durigan et al. 2010).

By using native forest species, the success of recovering degraded areas depends on the nutrition needs of each species during the seedling production in the nursery and during the establishment phase in the field aiming to increase the survival and growth potential. Nevertheless, studies have shown necessary in order to improve the production system for native forest species at a lower cost and with higher quality seedlings (José et al. 2005). It is a common concern for small landowners and nonprofit organizations that have a limited amount of resources to enable the production of seedlings as support for recovery.

The substrate is one of the factors that influence the growth and the quality of seedling production. To this, different mineral and organic materials can be used, even commercial substrates that can drive up costs of production (Monteiro et al. 2012). Because of this, the soil is an option of a substrate to small landowners and is generally used in a proportion approximated to 70% of container volume add to organic matter (Pozza et al. 2007).

Another kind of concern related to the seedling production system is the soil acidity. High levels of aluminum and manganese present in Brazilian soils cause this problem, in addition to low levels of calcium and magnesium that are essential nutrients for tree growth (Vieira et al. 2015).

However, soil correction by liming enables the neutralization and leaching of aluminum, and manganese charges, what can increase the base saturation of substrate and nutrient absorption. In addition, liming also helps to improve the availability of phosphorus in the soil, what benefits the development of root system, and the water and nutrients uptake from the substrate (Silva 2013). Despite the proven benefits of liming to a better growth of seedlings, the response of them is relative because each kind of crop presents a unique tolerance to soil acidity. (Bernardino et al. 2007).

Eugenia uniflora L. (pitangueira) and *Eugenia involucrata* DC. (cerejeira) are two native species that grow in the understory of the Mixed Ombrophilous Forest, and can be used in the recovering of degraded areas, urban forest, and urban park plantings (Lorenzi 2000; Sauressig 2014). Lorenzi (2000) and Sauressig (2014) stated that these species have a great ecological appeal due to their fruits that attract different kinds of animal species. However, to these species, studies about liming and fertilization are scarce, as in nursery as in pure or mixed plantings.

Due to the lack of basic information about the seedlings production system of *E. uniflora* and *E. involucrata* species, mainly to small producers, we aimed to test different dosages of dolomitic lime in a mixed substrate in order to determine which base saturation level would be the best one for the growth of both forest species.

Material and Methods

This research was done in the forest nursery of Universidade Estadual do Centro-Oeste, located in the city of Irati, Paraná State, Brazil. This city is covered by the Mixed Ombrophilous Forest ecosystem that is part of the Atlantic Forest Biome. The local climate is classified as Cfb by the Köeppen-Geiger classification, with severe and frequent frost in the winter and without a pronounced dry season.

We selected seedlings of *E. uniflora* L. (pitanga) and *E. involucrata* DC. (cerejeira) that had been produced in 100 cm^3 polyethylene tubes filled with the commercial substrate MaxFértil®, and the fertilizer Osmocote (14-14-14) in the proportion of 100 g per each 25 kg of substrate.

Selected seedlings had the same visual quality and similar total height, ranging from 8.0 cm to 11.0 cm. Four months after germination they were transplanted to 3.0-liter plastic bags filled with a substrate composed by a mixture of soil and sand in a 70/30 ratio because there was a high content of clay in the soil used (Table 1).

Table 1. Soil analysis results for chemical and physical characteristics

Variable	Value	Unity	Variable	Value	Unity
pH ($CaCl_2$)	4.1	---	Phosphorus	4.3	mg/dm^3
K^+	0.26	$cmol_c/dm^3$	Base saturation	35.8	g/100g
Ca^{2+}	3.6	$cmol_c/dm^3$	Thick sand	2.6	g/100g
Mg^{2+}	1.5	$cmol_c/dm^3$	Thin sand	2.9	g/100g
Al^{3+}	5.0	$cmol_c/dm^3$	Silt	40.5	g/100g
$H^+ + Al^{3+}$	9.63	$cmol_c/dm^3$	Clay	54.5	g/100g
Organic matter	40.9	g/dm^3			

Before transplanting, we added dolomitic lime to the composed substrate by mixing it, according to the amount related to each treatment. We used the commercial product Calfiller that has a relative neutralizing value equal to 85%, 20% of Ca^{2+} and 10% of Mg^{2+}. This limestone was applied in different dosages to increase the base saturation (V%) as different treatments:

Treatment 1 (T1) - Base saturation increased up to 40%;
Treatment 2 (T2) - Base saturation increased up to 45%;
Treatment 3 (T3) - Base saturation increased up to 50%;
Treatment 4 (T4) - Base saturation increased up to 55%;
Treatment 5 (T5) - Base saturation increased up to 60%.

We decided to test the base saturation influence from the initial value of 40% because the soil analysis showed a level of 35.8% to this variable. As we do not have information about the desirable base saturation values for these native forest species, and that to ornamental species the base saturation level must be up to 60% (Oleynik et al. 2004), we choose 60% as the uppermost value. In order to establish the base saturation values for each treatment, we used the V% equation recommended by Lima and Sirtoli (2006).

As a routine fertilization, six months after transplanting we applied 21.5 g of fertilizer per plastic bag to all treatments, based on phosphorus deficiency in the soil used as substrate, and on the best dosage of single superphosphate equal to 5.30 kg/m³ of substrate to the production of seedlings of *E. involucrata* (Mendonça et al. 2009).

For each species evaluated, treatments were displayed in a completely randomized design with five repetitions of three seedlings in each treatment. Twelve months after transplanting, we measured the total height and collar diameter of seedlings. We also estimated the height/collar diameter ratio (H/D).

In the statistical analysis, we used R software in order to apply Kolmogorov-Smirnov test to check normality, Bartlett test to check the homoscedasticity and the Analysis of Variance ($p<0.05$). When treatments were significantly different, we applied Tukey test to compare means of treatments. After this, we applied the regression analysis to check for changes in seedlings development promoted by the dosages tested, and the best value for each variable analyzed.

Results and Discussion

We observed a significant difference among treatments (Table 2) in relation to total height for *E. involucrata* (cerejeira) and total height, collar diameter and height/collar diameter ratio (H/D) for *E. uniflora* (pitangueira). In general, seedlings of both species were responsive to the increase of base saturation of substrate.

In a general way, the means of the coefficient of variation (CV) showed a small to medium variation (ranging from 16.21% and 22.21%), what is desirable in this kind of experiment that was taken outside de greenhouse and under local weather conditions. In the same manner, we must consider the natural genetic variability of seedlings, as they do not come from clonal material or improved trees. Pereira and Santana (2013) compared the coefficient of variation of 20 Fabaceae species from data about seed germination of high-quality plots. They found values ranging from 7.94% and 34.97% under controlled conditions of seed testing.

Table 2. Analysis of variance for total height, collar diameter, and H/D ratio of *Eugenia uniflora* (pitangueira) and *Eugenia involucrata* (cerejeira)

Source of variation	Degrees of freedom	Mean Square	Source of variation	Degrees of freedom	Mean Square
Total height for *Eugenia. uniflora*			Total height for *Eugena. involucrata*		
Factor	4	431,51 **	Factor	4	117, 75 **
Error	70	22,66	Error	70	16,22
Collar diameter for *Eugenia. uniflora*			Collar diameter for *Eugenia. involucrata*		
Factor	4	5,30 **	Factor	4	2,97 ns
Error	70	0,87	Error	70	1,42
H/D ratio for *Eugenia uniflora*			H/D ratio for *Eugenia involucrata*		
Factor	4	5,74 **	Factor	4	0,58 ns
Error	70	1,50	Error	70	0,81

** significant difference ($p < 0.01$)
ns not significant difference ($p > 0.05$)

By analyzing the effect of different levels of base saturation in the development of *E. uniflora* seedlings, we observed that treatment 2, corresponding to an increase of base saturation up to 45%, provided the best total height growth. We also observed the same results for *E. involucrata*.

Although the main effect first appears in the root system, seedlings total height can demonstrate the fastest reaction to the substrate quality (Silva et al. 2015). Thus, the fast growth of root system in the early stages can promote a good development of seedlings aboveground biomass. In this way, the substrate can be considered as the most important material to the quality of seedlings production, as long as it presents favorable features as good aeration, drainage, water retention and availability to the root system (Dutra et al. 2012).

There was a significant difference among treatments in relation to collar diameter for *E. uniflora*. The highest base saturation index (60%) differed from other treatments, but with the smallest effect on seedlings growth (Table 3). Although, the treatment two promoted the highest mean values for both species.

According to Binotto et al. (2010), collar diameter is the best indicator of quality because it has a strong relation to the survival rate and growth rhythm of seedlings after planting. With the exception of *E. uniflora* seedlings at the base saturation level of 60%, all the other treatments reached about 5.0 mm of collar diameter. This indicates that seedlings reached an acceptable stage of development, being suitable for planting by meeting the recommendations done by the authors.

Table 3. Descriptive statistics of height, collar diameter and H/D ratio for *Eugenia uniflora* (pitanga) and *Eugenia involucrata* (cerejeira) seedlings, with indication of the lowest (L), mean (M), highest (H), and the coefficient of variation (CV%) observed in each treatment

TOTAL HEIGHT (cm)				
Treatments	L	M	H	CV (%)
Eugenia uniflora				
T1	14.10	27.20 b	36.70	25.79
T2	28.90	35.84 a	42.80	13.16
T3	24.20	30.53 b	37.40	12.86
T4	21.30	28.88 b	37.50	13.79
T5	16.40	21.05 c	27.40	15.46
Mean	14.10	28.70	42.80	16.21
Eugenia involucrata				
T1	13.00	20.51 b	26.60	20.50
T2	22.60	26.75 a	32.20	11.60
T3	17.50	22.37 b	27.10	14.07
T4	10.30	20.29 b	28.10	25.20
T5	15.30	20.11 b	32.20	20.95
Mean	10.30	22.00	32.20	18.46

COLLAR DIAMETER (mm)				
Eugenia uniflora				
T1	2.46	5.14 a	7.07	22.90
T2	3.76	5.43 a	6.75	15.30
T3	3.42	4.63 ab	6.69	19.87
T4	3.49	4.83 a	6.41	17.46
T5	2.83	3.87 b	5.95	22.06
Mean	2.46	4.78	7.07	19.52
Eugenia involucrata				
T1	3.53	5.38 a	7.91	25.97
T2	5.20	6.27 a	8.12	14.10
T3	3.81	5.84 a	9.76	25.12
T4	3.64	5.28 a	6.88	19.56
T5	3.88	5.24 a	7.21	20.60
Mean	3.53	5.60	9.76	21.07
H/D RATIO				
Eugenia uniflora				
T1	2.93	5.40 b	7.97	22.83
T2	5.09	6.75 a	9.89	21.76
T3	5.26	6.73 a	8.69	15.87
T4	3.97	6.09 ab	8.34	17.28
T5	3.67	5.63 ab	7.20	22.44
Mean	4.18	6.12	8.42	20.04
Eugenia involucrata				
T1	2.69	3.92 a	5.28	19.12
T2	2.82	4.36 a	6.00	19.92
T3	2.09	4.03 a	6.67	26.91
T4	2.69	3.87 a	5.84	23.02
T5	2.77	3.92 a	6.16	22.09
Mean	2.61	4.02	5.99	22.21

Means followed by the same letter do not showed a significant difference ($p > 0.05$)

One of the main problems of a reduced development of collar diameter is the sustenance of an upright position after planting because it can promote a great probability of bending by means of wind. In this way, tall seedlings are not desirable if the development of collar diameter is inappropriate and H/D ratio must be considered as a variable to determine the quality of seedlings (Artur et al. 2007).

According to Grave et al. (2007), seedlings with a great collar diameter tend to show a more prominent development of total height, what is related to a good development of root system and the survival after planting. Ceconi et al. (2006) also state the same about the influence and correlation of collar diameter and total height of seedlings.

We observed a significant difference in the H/D ratio for *E. uniflora*, but only the treatment with the smallest level of base saturation (40%) differed from the others by means of the worst performance. However, as in the same way as for the variable diameter, treatment two promoted the highest mean value to H/D ratio.

According to Carneiro (1995), H/D ratio values between 5.4 e 8.1 can indicate a good quality pattern. Although, the development of seedlings depends on the environment control and their genetic characteristics. As shown in table 3, the mean values of H/D ratio were higher than this quality pattern in all treatments for *E. uniflora*.

The H/D ratio has been shown as a good parameter in the evaluation of the quality of forest seedlings because it can express a balanced relation of aboveground mass and root system, a better resistance (Sturion and Antunes 2000; Campos and Uchida 2002) and a better probability of survival after planting (Trautenmüller et al. 2016). Thus, seedlings can perform a high H/D ratio due to the increase of aboveground biomass during the first stages of growth (Silva et al. 2007).

In this way, the significant effect of liming over total height can indicate that the electrical charge correction improved the chemical features of the substrate. It happened because there was a low value for pH (4.1) and a very high value for aluminum (5.0 $cmol_c/dm^3$), in addition to a moderate content of Ca^{2+} (3.6 $cmol_c/dm^3$) and a high content of Mg^{2+} (1.5 $cmol_c/dm^3$) (Moreira et al. 2017).

The response to the gradual increase of base saturation suggests that *E. uniflora* and *E. involucrata* can perform a better height growth in limed soils. Even though these species can grow well at high natural acidity, low availability of exchangeable cations and high contents of aluminum, which are natural characteristics of soils in the Atlantic Rain Forest Biome (Vieira and Weber 2017).

Other native species show similar preferences for low levels of base saturation in the soil. Santin et al. (2013) observed that for *Ilex paraguariensis* a base saturation value between 30 and 45% promoted a great performance in total height and foliar area. Cruz et al. (2004) observed that a 50% value of base saturation promoted the greatest growth of total height, diameter, H/D ratio and biomass of *Tabebuia impetiginosa*.

According to Haridasan (2008), the efficiency of native plants during growth in poor soil conditions can be explained by a variety of mechanisms such as nocturnal root transpiration, nutrients cycling, and mycorrhizal association.

All the adjusted regression equations (Figure 1) presented coefficients of determination above 0.95. By the adjusted equations, it is possible to assume that to increase the growth of *E. uniflora* and *E. involucrata* the best base saturation value must be approximately to 48%.

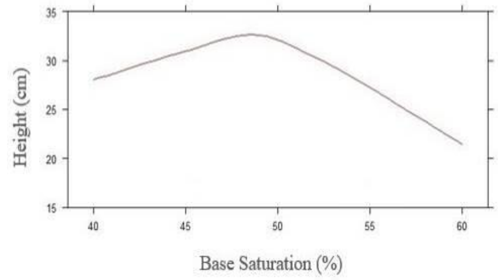
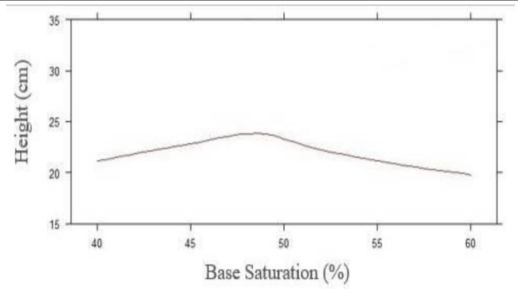
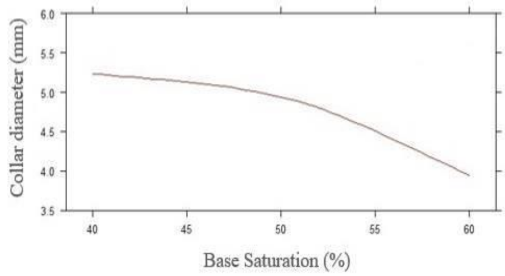
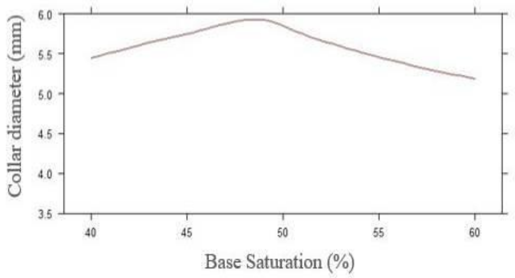
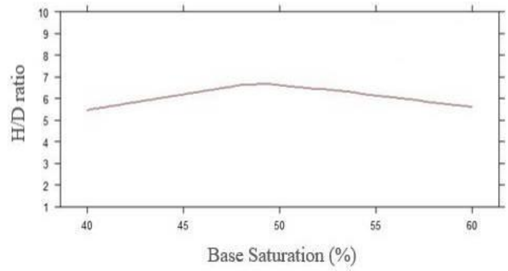
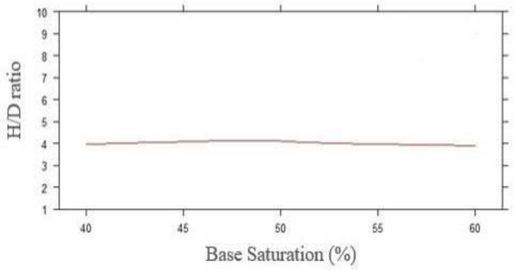
	Eugenia uniflora	*Eugenia involucrata*
Total Height (cm)		
	$y = -1623x + 10030x^2 - 19280x^3 + 11950x^4$ ($r^2 = 0.9713$)	$y = -2059x + 12931x^2 - 25929x^3 + 16983x^4$ ($r^2 = 0.9669$)
	p-value of coefficients β_0: 0.0242; β_1: 0.0188; β_2: 0.0235; β_3: 0.0338	p-value of coefficients β_0: 0.0007; β_1: 0.0040; β_2: 0.0003; β_3: 0.0004
Collar diameter (cm)		
	$y = 47.1x - 171.1x^2 + 301.6x^3 - 214.1x^4$ ($r^2 = 0.9618$)	$y = -303.2x + 1953x^2 - 3932.3x^3 + 2573.3x^4$ ($r^2 = 0.9572$)
	p-value of coefficients β_0: 0.0063; β_1: 0.0078; β_2: 0.081; β_3: 0.0081	p-value of coefficients β_0: 0.0499; β_1: 0.0460; β_2: 0.0331; β_3: 0.0350
H/D ratio		
	$y = -135.6x + 919x^2 - 1891.6x^3 + 1258.1x^4$ ($r^2 = 0.9622$)	$y = -323.3x + 2007.3x^2 - 3897.7x^3 + 2460.3x^4$ ($r^2 = 0.9528$)
	p-value of coefficients β_0: 0.0341; β_1: 0.0225; β_2: 0.0491; β_3: 0.0359	p-value of coefficients β_0: 0.0112; β_1: 0.0247; β_2: 0.0341; β_3: 0.0221

Figure 1. Regression analysis developed from data about height, collar diameter and H/D ratio of *Eugenia uniflora* and *Eugenia involucrata* seedlings based on the increase of base saturation in the substrate

Base saturation levels greater than 50% decreased height and collar diameter growth of *E. uniflora* and *E. involucrata* as the same manner as to *Tabebuia impetiginosa* (Cruz et al. 2004) and *Ilex paraguariensis* (Santin et al., 2013). High levels of base saturation can induce an unbalance in the Ca:Mg ratio in the soil, what can compromise seedlings development (Salvador et al. 2011).

Nonetheless, other species have a better growth in different base saturation levels. Vieira et al. (2015) observed that a base saturation level greater than 60% provided the best height and biomass growth in seedlings of *Amburana acreana*, while Vieira and Weber (2017) concluded that base saturation levels greater than 70% were the best ones for *Tabebuia serratifolia* seedlings.

Conclusions

The increasing of base saturation in the substrate influenced height, collar diameter, and H/D ratio of *E. uniflora* and *E. involucrate* seedlings.

The best treatment was the one with a base saturation in the level of 45%, although regression analysis revealed that a level of 48% could promote greater growth for both species.

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