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Cálculo do tamanho ótimo de parcela para experimentos com eucalipto

Calculation of the ideal plot size for experiments using eucalyptus

Cálculo del tamaño óptimo de parcela para experimentos con eucalipto

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Resumo

Um planejamento experimental adequado depende de vários fatores, como homogeneidade da área e unidade experimental, número de repetições e tamanho da parcela. A adoção de um tamanho ideal de parcela reduz o erro experimental e, consequentemente, maximiza as informações obtidas em um experimento. Assim, o objetivo do presente estudo foi calcular o tamanho ótimo de parcela da cultura de eucalipto em casa de vegetação, a fim de otimizar mão de obra e custos no processo de cultivo dessa cultura economicamente importante. Para calcular o tamanho ótimo de parcela, várias simulações foram realizadas usando o método de curvatura máxima modificada. Ao longo do período experimental, foram utilizadas mudas clonais do híbrido *Eurograndis*, que foram avaliadas quanto à altura (H), diâmetro do caule na base (DC), teor de clorofila nas folhas velhas (Clor V) e folhas novas (Clor N) aos 30, 60, 90 e 120 dias após o transplante de mudas. Após 120 dias, as mudas foram coletadas para determinação da massa seca da parte aérea (MSPA) e do sistema radicular (MSR). Os resultados indicaram que o tamanho ideal recomendado para o eucalipto, em estufa, foi de 6 plantas por parcela.

Palavras-chave: Variação experimental; Precisão experimental; Curvatura máxima modificada.

Abstract

An adequate experimental planning depends on several factors, such as homogeneity of the area and experimental unity, number of repetition and plot size. The adoption of an ideal plot size reduces the experimental error and, consequently, maximizes the information obtained in an experiment. Thus, the aim of the present study was to calculate the optimal plot size of eucalyptus culture in a greenhouse, in order to optimize labor and costs in the cultivation process of this economically important culture. To calculate the optimal plot size, several simulations were performed using the modified maximum curvature method. Throughout the experimental period, clonal seedlings of the *Eurograndis* hybrid were used and evaluated for height (H), stem diameter at the base (DB), chlorophyll content in old leaves (Chlor V) and new leaves (Chlor N) at 30, 60, 90 and 120 days after seedling transplantation. After 120 days, the seedlings were collected to determine the dry mass of the aerial part (DMAP) and the root system (DMRS). Results indicated that the optimal recommended plot size for eucalyptus, in a greenhouse, were 6 plants per plot.

Keywords: Experimental variation; Experimental precision; Maximum modified curvature.

Resumen

Una planificación experimental adecuada depende de varios factores, como la homogeneidad del área y la unidad experimental, el número de repeticiones y el tamaño de la parcela. La adopción de un tamaño de parcela ideal reduce el error experimental y, en consecuencia, maximiza la información obtenida en un experimento. Por lo tanto, el objetivo del presente estudio fue calcular el tamaño óptimo de parcela del cultivo de eucalipto en un invernadero, con el fin de optimizar la mano de obra y los costos en el proceso de cultivo de este cultivo económicamente importante. Para calcular el tamaño óptimo de la parcela, se realizaron varias simulaciones utilizando el método de curvatura máxima modificado. A lo largo del período experimental, se utilizaron plántulas clonales del híbrido *Eurograndis*, que se evaluaron para la altura (H), el diámetro del tallo en la base (DB), el contenido de clorofila en las hojas viejas (Clor V) y las hojas nuevas (Clor N) a los 30, 60, 90 y 120 días después del trasplante de plántulas. Después de 120 días, se recogieron las plántulas para determinar la masa seca de la parte aérea (MSPA) y el sistema de raíces (MSR). Los resultados indicaron que el tamaño óptimo de parcela recomendado para eucalipto, en un invernadero, fue de 6 plantas por parcela.

Palabras clave: Variación experimental; Precisión experimental; Curvatura máxima modificada.

1. Introduction

Eucalyptus is an increasing culture of significant relevance in the national economic context. The eucalyptus cultivation area has been increasing year by year in Brazil, and it represented 75% of the 10 million hectares of forests planted in 2016 (NFIS, 2017). Despite having appropriate area and conditions for the highest development of this culture in this country, there is a need of research to support improvements for implementation, management, production and consequently a higher supply to the market.

A significant limitation to the study of several crops has been the adequate definition of the size and number of plots. Nowadays, it is difficult to find data in the literature related to a constant number of plants per plot, which makes the application of usual procedures for data analysis not feasible (Ramalho et al., 2012). Therefore, experiments may be performed with unnecessary labor and costs in the implementation, making the ideal plot size a procedure of greatest importance.

To give credibility to the research it is important to evaluate the data using statistical instruments that ensure greater consistency. The researcher is responsible to determine and control all factors interfering in the experiment, making the research to be more accurate and of high reliability. The determination of the size of the plots in the agricultural experimentation is related to the reduction of the experimental error, due to the heterogeneity of the plots (González, 2013).

The experimental error is the variation between the plots, which, by removing the possible effects of control, can be minimized, but not totally eliminated. The fundamental method to avoid heterogeneity is the use of an appropriate experimental design, number of repetitions, plot size and shape, and number of treatments with the required precision for the experiment (Storck et al., 2011).

Although crops usually present high survival rates, for a good development of the plant in the field, seedlings of good quality are necessary, and this is an extremely important aspect for success in forest stands (Ferraz & Engel, 2011; Silva et al., 2012). Several factors influence the seedling development process, such as nutrition, soil and irrigation (Costa et al., 2011). A method to minimize deleterious effects is to perform the experiment with protected crops, controlling the effects, improving the quality of the seedlings, and consequently, the production.

In this context, the determination of an ideal plot size for field and greenhouse experiments, for eucalyptus cultivation, contributes to perform experiments with higher precision, maximizing the obtained information, decreasing the waiting time in obtaining satisfactory results to the implementation of the crop, ensuring greater effectiveness and efficiency of the production process.

Thus, this study aimed at defining the ideal size of experimental plots for experiments with eucalyptus in greenhouses, using the method of maximum modified curvature, in order to optimize labor and costs in the cultivation process of this economically important culture.

2. Methodology

To determine the ideal plot size, the experiment was conducted as a blank test with 100 repetitions with clone seedlings of the hybrid VR3 *Eucalyptus grandis* x *Eucalyptus urophylla*, called *E. urograndis,* under protected culture conditions (greenhouse), at UNESP/Campus de Ilha Solteira, SP, Brazil, from Febuary to June 2014.

The soil used in the experiments was collected at a depth of 0.0 to 0.20 m, under eucalyptus plantation, located in the municipality of Três Lagoas, MS, Brazil (Latitude: 20º 59'S and Longitude 51º 48' O). Soil samples were sieved (4mm) and packed in bags for seedlings with a height of 0.40 m (8 kg of soil). Limestone and NPK (Nitrogen, Phosphorus and Potassium) were incorporated to the soil, according to the regional recommendation for eucalyptus culture (1.5 Mg/ha of limestone, 0.5 Mg/ha NPK - 12-20-16). Eucalyptus seedlings (*Eucalyptus* spp.), used as indicators, were donated by FIBRIA Ltda.

During the experimental period, the irrigation was performed with purified water (reserve osmosis), controlled according to the water retention capacity of the soil (Silva et al., 2017), to prevent leaching. The soil fertility and texture were evaluated according to Raij et al. (2001) and Teixeira et al. (2017), before the beginning of the experiment, and results indicated a sandy soil of low fertility (Table 1).

Table 1. Fertility and texture of the soil.

Fertility											Texture*	
MO	P	K	Ca	Mg	H+Al	Al	CTC	V	m	pH	Sandy	98
(g/dm³)	(mg/dm³)	(mmol$_c$/dm³)						(%)			Silt	874
27	6	1,3	13	9	58	7	81,3	29	23	4,2	Clay	28

*(g kg^{-1}). Source: Authors.

During the experimental period, eucalyptus seedlings were evaluated for: height (H) in centimeters, with a millimeter ruler; stem diameter at the base (DB) in millimeters, with a digital pachymeter MTX 150 mm; chlorophyll content in old (Chlor V) and new (Chlor N) leaves, in Falker Chlorophyll Index (FCI) with ChlorofiLOG (Falker Agricultural Automation, Brazil). All variables were measured at 30, 60, 90 and 120 days from the transplant of the seedlings. After 120 days, the seedlings were collected to determinate the dry mass of the aerial part (DMAP) and the root system (DMRS) obtained after drying in circulation greenhouses.

To calculate the optimal plots size, 31 simulations of different forms of plots were performed, using the method of maximum modified curvature in the uniformity test. For all studied variables, coefficients of variation were calculated for each plot size based on different shapes, corresponding to 17 plot sizes, with the size of the basic unit (bu) ranging from 1 to 50 plants and the plot number from 2 to 100 (Table 2). For simulated plots of the same size, but with different shapes, the average coefficients of variation for H, DB, Chlor N, Chlor V, DMAP and DMRS were calculated.

The ideal plot size was calculated by the method of maximum modified curvature as proposed by Lessman & Atkins (1963). By this method, the relation between the coefficient of variation (CV) and the plot size with X basic units is explained by the model

$$CV = aX^{-b}$$

where a and b are the parameters to be measured. From the curvature function given by this model, the value of the abscissa was determined, where the point of maximum curvature occurs, given by:

$$X_0 = \left[\frac{a^2 b^2 (2b+1)}{b+2}\right]^{\frac{1}{2b+2}}$$

The X_0 is the value of the abscissa at the point of maximum curvature, corresponding to the estimate of the ideal size of the experimental plot (Meier & Lessman, 1971).

The statistical method t test ($p \leq 0.001$) was applied to test the hypotheses related to the parameters of the model used.

Table 2. Number of simulations (NS), number of plots (NP), plot size (PS) and plot shape (PSS) for the basic units of experiments with eucalyptus in the greenhouse.

NS	NP	PS	PSS	NS	NP	PS	PSS	NS	NP	PS	PSS
1	100	1	1x1	12	10	7	2x3+1	23	3	18	3x6
2	49	2	2x1	13	9	7	3x2+1	24	3	18	6x3
3	49	2	1x2	14	10	8	2x4	25	4	20	4x5
4	30	3	3x1	15	10	8	4x2	26	4	20	5x4
5	30	3	1x3	16	10	10	2x5	27	4	25	5x5
6	25	3	2+1	17	10	10	5x2	28	2	30	5x6
7	25	3	1+2	18	6	12	3x4	29	2	30	6x5
8	25	4	2x2	19	6	12	4x3	30	2	50	5x10
9	15	5	2x2+1	20	6	15	3x5	31	2	50	10x5
10	15	6	2x3	21	6	15	5x3				
11	15	6	3x2	22	4	16	4x4				

1x1 is read: one value of the line by one value of the column; 2x1 is read: two values of the line are added together in each column; 2+1 is read: two values of the line are added together by adding one value of the column; 2x2+1 is read: sum of two values of the line, two values of the column by adding one more unit. Source: Authors.

The statistical analyses related to the method used, as well as the illustrative charts were performed using the software R version 3.5.1 (R development core team, 2018).

3. Results and Discussion

Regardless the variables, the highest CV values found were for 1 bu (plants per plot). The variation registered for the highest and lowest CV value, considering all variables studied and the respective plot size was 36.04. The lowest value (0.62) was found for the variable ChlorV after 30 days in the plot with 50 bu, and the highest value (36.66) was found for DMAP, after 120 days in the plot with 1 bu.

For Chlor V, the value obtained the CV for the smallest basic unit (1bu) was equivalent to 10% for DB, 15% for Chlor N, 25% to for H, in all evaluations performed. For DMRS and DMAP this value was equal and above 30%, respectively.

Since there is no specific coefficient of variation classification for eucalyptus, the Pimentel-Gomes classification (2009) was used. According to this classification, Chlor V presented low variation, DB, Chlor N and H presented medium variation, and DMAP and DMRS presented high variation. The values found for the largest basic unit (50 bu) were 6% for H, 4% for DB, 2% for Chlor V, 5% for Chlor N and DMRS and 8% for DMAP. All values were adjusted to the range of low variation.

According to Cargnelutti Filho et al. (2018), experimental coefficients of variation (CVe) lower than 10% guarantee high precision to the experiment. Following this premise, only 4 bu (plants per plot) would be necessary for the DB, Chlor V and N, 6 bu for H, 18 bu for DMRS and 30 bu for DMAP variables. However, according to the obtained results, when using larger plots there was a gain in precision in all the variables analyzed, justifying the need to calculate the ideal plot size for each variable, respecting the nature of data, as one cannot be at risk of underestimating or overestimating the ideal size.

All parameters of the model found for the variables analyzed were statistically significant according to the t test ($p<0.001$). For the variables H, DMAP, DB, and DMRS, the standard errors of the estimates (Table 3) were low for all evaluations, promoting more significant results.

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Table 3. Parameters estimates for the method of maximum modified curvature and standard error of estimate (S.E.E.) for the t-test applied for variables H, DB, DMAP and DMRS.

Variables	H								DMAP	
Evaluation	30		60		90		120		120	
Parameters	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$
Estimates	19.44	0.40	22.45	0.63	21.35	0.58	25.29	0.61	37.00	0.36
S.E.E	1.07	0.03	1.04	0.04	0.64	0.22	0.78	0.02	1.32	0.03
Variables	DB								DMRS	
Evaluation	30		60		90		120		120	
Parameters	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$
Estimates	13.87	0.42	12.50	0.41	12.56	0.45	14.01	0.43	30.31	0.45
S.E.E	0.49	0,02	0.53	0,25	0.53	0,03	0.63	0,03	1.32	0,03

For all variables the p-value was less than 0.001 (Statistically significant by t-test). Source: Authors.

Table 4 shows the results of the analysis for the variables Chlor V and Chlor N; the standard errors of the estimates were also low for all evaluations, according to the t test ($p < 0.001$). The values for the standard error were always lower than the estimates throughout the evaluation period, when considering all studied variables (Tables 3 and 4). Since the standard error calculates the reliability on which the estimates were calculated, it is possible to conclude that the modified maximum curvature model presented satisfactory results for all variables evaluated.

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Table 4. Estimates of the parameters for the modified maximum curvature method and standard error of the estimate (S.E.E.) for the t test, applied to the variables Chlor V and Chlor N.

Variable	Chlor V							
Evaluation	30		60		90		120	
Parameters	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$
Estimatives	9, 8	0.54	7.56	0.47	11.50	0.59	10.29	0.43
S.E.E.	0.33	0.02	0.63	0.05	1.12	0.07	0.99	0.06
Variable	Chlor N							
Evaluation	30		60		90		120	
Parameters	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$	$\hat{a}$	$\hat{b}$
Estimatives	12.34	0.33	12.97	0.48	15.58	0.44	12.84	0.36
S.E.E.	0.95	0.04	0.62	0.03	1.06	0.04	0.91	0.04

For all variables the p-value was less than 0.001 (Statistically significant by t-test). Source: Authors.

For all variables analyzed, high values of coefficient of determination were obtained, indicating a good precision of the model (Table 5) (Lúcio et al., 2015).

The mean values of coefficient of determination (R^2) varied from 90% to 98% for height, 93% to 96% for diameter, 81% to 97% for Chlor V, 83% to 94% for Chlor N, 95% for DMAP and 94% for DMRS (Table 5, Figure 1 and 2). Since the R^2 determines the degree of adjustment of the methodology used (Ferreira, 2018), the applied model explained at least 80% of the variation. However, for most variables, values above 95% were registered, being only 5% not explained by the model, indicating that data were adjusted to the modified maximum curvature method (MMCM).

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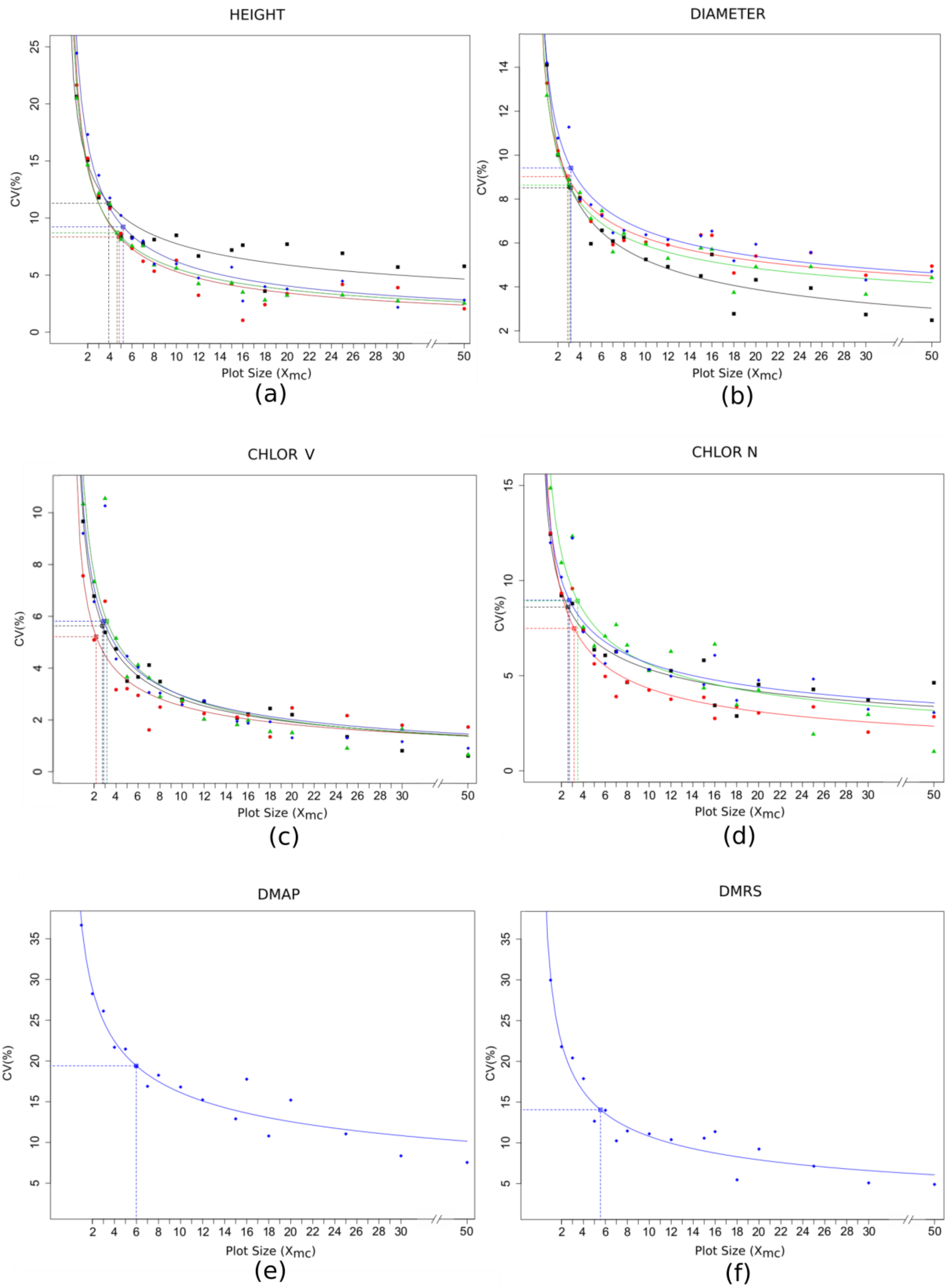
Figure 1. Values obtained for the plot sizes of variables H, DB, CHLORV and CHLORN at 30 (curve in black), 60 (curve in red), 90 (curve in green) and 120 (curve in blue) days, and for the variables DMAP and DMRS at 120 days.



Source: Authors.

For variable H, the plot sizes found ranged from 3.90 (30 days) to 5.20 (120 days) (Figure 1 a), and a plot size of 5 bu (plants per plot) was indicated as appropriate. For DB there was a variation from 2.88 (60 days) to 3.18 (120 days) (Figure 1 b), suggesting a plot size of 4 bu. For the variable Chlor V, plot sizes ranged from 2.19 (60 days) to 3.16 (90 days) (Figure 1 c). A ranged from 2.61 (30 days) to 3.49 (90 days) was found for the variable Chlor N, (Figure 1 d). For the variables DMAP and DMRS, the plot sizes found at 120 days were 5.98 (Figure 1 e) and 5.55 (Figure 1 f), indicating the 6 bu size plots as adequate (Table 5).

Table 5. Coefficients of determination (R^2), coefficient of variation corresponding to the point of maximum curvature (P) and estimates of ideal plot sizes (X_{MC}) as a function of the evaluation periods for variables H, DB, Chlor V, Chlor N, DMAP and DMRS.

Variables	H				DB				DMAP
Evaluation	30	60	90	120	30	60	90	120	120
P	11.33	8.35	8.71	9.23	8.52	8.11	7.56	8.56	19.4
X_{MC}	3.90	4.85	4.65	5.2	3.16	2.88	3.02	3.18	5.98
R^2	0.90	0.96	0.98	0.98	0.96	0.93	0.95	0.94	0.95
	ChlorV				ChlorN				DMRS
Evaluation	30	60	90	120	30	60	90	120	120
P	5.63	5.21	5.81	5.81	8.6	7.48	8.93	897	14.1
X_{MC}	2.77	2.19	3.16	2.87	2.61	3.17	3.49	2.73	5.55
R^2	0.97	0.82	0.83	0.81	0.89	0.94	0.88	0.83	0.94

*H: height; DB: diameter; DMAP: dry mass of the aerial part; ChlorV: chlorophyll in old leaves; ChlorN: chlorophyll in new leaves; DMRS: dry mass of the root system. Source: Authors.

According to the obtained results, it is possible to conclude that the recommendation for the optimal size plot was 6 bu (plants per plot), based on the minimum PS required for all studied variables. The same plot size was used in the field by Bartieres et al. (2016) and Araújo et al. (2015).

Underestimated values were found in Matos et al. (2018) and Bellé et al. (2018), which used one plant per plot, and Caldeira et al. (2013), which used 4 plants per plot. We believe that those data would not be sufficient to ensure accuracy of the experiment, since the information was minimized and no satisfactory results could be obtained.

On the other hand, overestimated values were used by Magalhães et al. (2017) and Silva et al. (2016), with 16 and 10 plants per plot, respectively, being 10 and 4 bu in sequence. According to our findings, 6 plants per plot would be enough, and this size of plot could decrease the costs of the experiment, achieving the same precision as larger plots.

Despite no studies were found in literature for PS of eucalyptus in greenhouses, some authors studied PS of eucalyptus in the field, using three different methods: the recommendation of 4 bu (Muniz et al. 1999), 5 bu (Muniz et al., 2009) and 10 bu (Simplício et al., 1996), by the method of Hatheway & Williams (1958); of 10 bu (Zanon & Storck, 2000) by the maximum curvature method, and of 5 bu (Silva et al. 2003) by the modified maximum curvature method.

Cargnelutti Filho et al. (2011), Morais et al. (2014), Padrón et al. (2018) and Santos et al. (2018), used the method of maximum modified curvatures for maize, tissue vines, pepper and lettuce cultures, while Rodríguez et al. (2018) used the same method for sweet potato culture, demonstrating that this is an well established method.

With the modified maximum curvature model, it is possible to reduce experimental errors and ensure greater precision (Cargnelutti Filho et al., 2018). Through this method, the estimates found with eucalyptus in the field, 5 bu (Silva et al., 2003) and those obtained in this research, in a 6 bu greenhouse were very close, showing that the plot size in a vegetation house will not be so far from the value considered ideal for installation in the field.

Planting under protected cultivation becomes advantageous, since it is possible to control climatic factors and/or pests that may interfere with the growth of the plant, as well as luminosity and temperature that may also interfere with their production (Costa et al., 2010). It was observed, by means of blank tests under protected cultivation, that it was possible to know the behavior of the plants and, after the analysis, that they could go through several periods of acclimatization, until being discarded in the field. Knowing the behavior of the plant is extremely important, to obtain greater experimental accuracy, because one can understand all the characteristics that need to be controlled, since they can interfere directly or indirectly in the optimal development of the plants.

4. Conclusion and Suggestions

The ideal plot size depends on the variables, being 6 plants per plot for height, 4 plants per plot for diameter, Chlor V and Chlor N, 6 plants per plot for DMAP and DMRS.

Based on the ideal minimum plot size including all variables, it is recommended the ideal plot size with eucalyptus cultivation under greenhouse of 6 plants per plot.

In future studies, other methods may be added and research related to the ideal number of repetitions may also be carried out.

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