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Research Article

Optimal plot size with conilon LB1 coffee tree clonal seedlings produced in tubes¹

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ABSTRACT

In experimental studies, achieving an optimal plot configuration holds significant importance. This study aimed to establish suitable experimental plot sizes for evaluating agronomic traits of clone LB1 conilon coffee seedlings grown in tubes. Both non-destructive and destructive traits were evaluated using seedlings obtained from a nursery, with each seedling allocated to basic experimental units. A completely randomized block design was used and various scenarios were generated based on combinations of treatments (3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35 and 40), replicates (3, 4, 5, 6 and 7) and levels of experimental precision (10, 20, 30 and 40 %). Convenient plot size recommendations were obtained for clone LB1 conilon coffee seedlings grown in tubes.

KEYWORD: Coffea canephora Pierre ex A. Froehner, Rubiaceae, experimental accuracy.

INTRODUCTION

Conilon coffee (*Coffea canephora* Pierre ex A. Froehner), a member of the Rubiaceae family, holds significant national and international importance. In 2022, the Espírito Santo state produced around 12.4 million bags of conilon coffee (Conab 2022), positioning the state at the forefront of the national production.

The LB1 clone, one of numerous clones derived from the *Coffea canephora* species, is extensively used by several rural producers in Espírito Santo and certain regions of Bahia. It demonstrates compatibility with other clones used in clonal varieties and is notable for its characteristic corolla

RESUMO

Tamanho ótimo de parcela com mudas clonais de cafeeiro conilon LB1 produzidas em tubetes

Em estudos experimentais, alcançar uma configuração ideal de parcela é de importância significativa. Objetivou-se estabelecer tamanhos de parcelas experimentais adequados para a avaliação de características agronômicas de mudas do clone LB1 de café conilon cultivadas em tubetes. Tanto as características não destrutivas quanto as destrutivas foram avaliadas a partir de mudas obtidas em viveiro, sendo cada muda alocada em unidades experimentais básicas. Utilizou-se delineamento de blocos inteiramente casualizados e vários cenários foram gerados com base em combinações de tratamentos (3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35 e 40), repetições (3, 4, 5, 6 e 7) e níveis de precisão experimental (10, 20, 30 e 40 %). Obtiveram-se recomendações convenientes de tamanho de parcela para mudas do clone LB1 de café conilon cultivadas em tubetes.

PALAVRAS-CHAVE: *Coffea canephora* Pierre ex A. Froehner, Rubiaceae, precisão experimental.

tube length (Silva et al. 2021), which influences the pollination process. The LB1 genotype is included in the Monte Pascoal cultivar, listed in the national register of cultivars of the Brazilian Ministry of Agriculture, Livestock and Supply (Brasil 2024). Renowned for its productivity, the Monte Pascoal cultivar yields an average of 130 bags ha⁻¹ year⁻¹ (Partelli et al. 2021). This figure exceeds the average Brazilian production of conilon coffee, which reached 46.8 bags ha⁻¹ in 2022 (Conab 2022).

Regarding seedling production, the use of tubes (a rigid container made of polypropylene) facilitates the seedling transportation and enhances the phytosanitary quality and planting yield (Mauri et al. 2015). Additionally, they can increase the

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production and biomass accumulation of conilon coffee seedlings, when compared to the use of plastic bags (Verdin Filho et al. 2021).

The literature outlines several methods for determining plot size, including the modified maximum curvature method, as per Meier & Lessman (1971), and the Hatheway's convenient size method (1961). The Hatheway's method considers the experimental design, number of treatments, number of replications and desired experimental precision. This approach enables to select an optimal plot size combination, number of replications and level of experimental precision (Magalhães et al. 2023).

Experimental design and plot size are typically determined based on the researcher's expertise and the human and financial resources available for conducting the experiment (Guarçoni et al. 2020). The literature presents evaluations of conilon coffee seedlings using tubes with varying quantities, such as 3 (Amaral et al. 2007), 12 (Silva et al. 2010) and 15 seedlings plot⁻¹ (Espindula et al. 2018).

Ideally, the optimal plot size should be based on scientific rationale to maximize resource and input utilization. Thus, this study aimed to determine optimal experimental plot sizes for clone LB1 conilon coffee seedlings cultivated in tubes using the Hatheway's method (1961).

MATERIAL AND METHODS

Conilon coffee seedlings were acquired on April 5, 2018, from a nursery located in the municipality of Jaguaré, in the northern region of the Espírito Santo state, Brazil (-18.90369533560803°S and -40.04206438839696°W). In total, this study analyzed 108 seedlings divided into two trays, each containing 54 tubes with volume of 250 cm³.

A polyethylene mesh (Sombrite®) was employed to allow 50 % of sunlight penetration into the experimental area, ensuring the protection and coverage of the seedlings. The irrigation system used the micro-spray technology. The seedlings were propagated from cuttings of orthotropic branches, with approximately 10 cm in length, retaining a pair of leaves on each cutting.

Following staking, the seedlings were transferred after 90 days to the plant breeding laboratory of the Universidade Federal do Espírito Santo. Non-destructive traits were recorded, including shoot height (SH), measured in centimeters using a

graduated ruler from the stem base to the insertion of the last leaf; number of leaves, determined by counting all leaves of each seedling; collar diameter (CD), measured in millimeters using a digital caliper at 3 cm from the substrate surface; and leaf area per seedling, scanned with a Vupoint® Solutions Magic Wand portable scanner (PDS-ST415-VPS) at 75 dpi in .TIFF format, a non-destructive method preferred for its accuracy (Santos et al. 2023), and analyzed using the ImageJ® software (Schindelin et al. 2015).

Subsequently, the seedlings were rinsed under running water to remove the substrate. The tops and roots of each seedling were separated, placed in a forced ventilation oven at 60 °C, and kept until reaching a constant mass. This process enabled the assessment of destructive traits, including the shoot dry matter (SDM), in grams; total dry matter (TDM), in grams; root dry matter (RDM), obtained by subtracting the shoot dry matter from the total dry matter; and the Dickson's seedling quality index (DQI), calculated using the following equation (Dickson et al. 1960): DQI = TDM/[(SH/CD) + (SDM/RDM)].

Using the Hatheway's methodology (1961), based on the evaluation of eight agronomic traits, the appropriate size of the experimental plots was determined. A total of 108 seedlings from the blank test were organized into basic experimental units, comprising 9 rows of 12 seedlings each, with each basic experimental unit containing one seedling. The basic experimental units were grouped according to the exact divisors of the total number of seedlings from the blank test, resulting in ten groupings. For each grouping, all possible combinations for evaluating the eight traits of the seedlings were considered. The cluster sizes are presented in Table 1.

For each Xi basic experimental unit, the following parameters were calculated: $m_{(Xi)}$: average of the Xi basic experimental units; $V_{(Xi)}$: variance between plots and Xi basic experimental units sizes; $CV_{(Xi)}$: coefficient of variation between plots and Xi basic experimental units sizes; $VU(Xi) = V_{(Xi)}/X^2$: variance per basic experimental unit between plots and Xi basic experimental units sizes. The heterogeneity index (b), based on the logarithm of the function to the base 10 in the equation $VU(Xi) = V_{(i)}/X_1^b$, according to Smith (1938), was determined by the ratio of $VU_{(Xi)}$ as a function of Xi.

For each characteristic, the optimal plot size (X_0) for the experimental design, in terms of the

Table 1. Projected plot size (X_i = BR x WR) into basic experimental units, plot format (BR = between rows of nine tubes; WR = within rows of nine tubes) and total number of plots for groupings in a blank test of clone LB1 conilon coffee seedlings (*Coffea canephora* Pierre ex Froehner) in tubes to determine the Smith's heterogeneity index.

Grouping	Size plot (X_i)	Plot format	Plot number
1	1	(1 x 1)	108
2	2	(2×1)	54
3	3	$(3 \times 1); (1 \times 3)$	36
4	4	(4×1)	27
5	6	$(6 \times 1); (2 \times 3)$	18
6	9	$(1 \times 9); (3 \times 3)$	12
7	12	(12×1)	9
8	18	$(6 \times 3); (2 \times 9)$	6
9	27	(3×9)	4
10	54	(6 x 9)	2

number of basic experimental units, was calculated using the Hatheway's method (1961), as given by the equation $X_0 = \sqrt[6]{[2(t_1 - t_2)^2 \text{ CV}^2]/\text{rd}^2}$, where b represents the Smith's heterogeneity index; CV the estimated coefficient of variation between the plots of a single basic experimental unit size, in percentage; r the number of considered replications; d the difference between treatment means to be detected as significant at a 5 % probability, expressed as a percentage of the overall mean of the uniformity test; t, the tabulated value of t for significance tests (bilateral test at 5 %) with df degrees of freedom; t, the bilateral tabulated value of t, corresponding to an error of 2(1-p) with df degrees of freedom, where p = 0.80 represents the probability of obtaining significant results. The tabulated values of the t distribution were obtained with df degrees of freedom of residue as a function

of I treatments and r replications, where df = (I - 1) (r - 1) for randomized block experiments. Basic experimental units of the same size Xi and different shapes were grouped to facilitate the analyses.

The Hatheway's method (1961) yielded scenarios for each evaluated trait in the randomized block design. The scenarios were generated by combining treatments (I = 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35 and 40), replications (r = 3, 4, 5, 6 and 7) and differences (d = 10, 20, 30 and 40%) between the treatment means to be detected as significant at 5% of probability, with results expressed as percentage of the overall mean of the uniformity test.

The analysis revealed that each trait may require a different plot size (X_0) . Consequently, combining several traits resulted in various plot sizes. Among the evaluated traits, X_0 was defined based on the highest CV value in the sample. X_0 was determined for one non-destructive characteristic with the highest CV and one destructive characteristic also with the highest CV.

The data were analyzed using the R software (R Development Core Team 2023). As the optimal plot size is a discrete random variable, it was presented as an integer and rounded up to the nearest whole number (Celanti et al. 2016).

RESULTS AND DISCUSSION

This study evaluated seedlings under shipping conditions from the nursery and means obtained for traits that were either similar to or higher than those found in other studies. For example, Table 2 indicates that the means for shoot height and root dry mass were comparable to those reported by Meneghelli

Table 2. Descriptive statistics showing minimum, maximum and mean values, standard deviation (SD), coefficient of variation (CV) and p-value of the Shapiro-Wilk (SW) normality test for 108 clonal conilon coffee seedlings (*Coffea canephora* Pierre ex Froehner), clone LB1, assessed at 90 days after staking.

Characteristic ¹	teristic ¹ Minimum		Mean	SD	CV (%)	SW ²				
Non-destructive										
SH	13.50	26.60	21.21	2.80	13.18	> 0.01				
CD	2.66	4.71	3.72	0.42	11.36	0.38				
NL	6.00	15.00	9.28	1.90	20.47	< 0.01				
LA	184.95	413.14	284.61	49.09	17.25	> 0.01				
	Destructive									
SDM	2.07	6.64	3.19	0.67	20.96	< 0.01				
RDM	0.46	3.54	1.35	0.44	32.63	< 0.01				
TDM	2.64	8.85	4.55	0.91	20.00	< 0.01				
DQI	0.26	1.22	0.57	0.17	30.13	< 0.01				

¹ SH: shoot height (cm); CD: collar diameter (mm); NL: number of leaves per plant; LA: leaf area (cm²); SDM: shoot dry mass (g); RDM: root dry mass (g); TDM: total dry mass (g); DQI: Dickson's quality index. ² p-value higher than 0.05 indicates normal data distribution according to the Shapiro-Wilk normality test.

et al. (2018). The collar diameter, a crucial indicator of seedling quality and survivability, closely resembled measurements reported by Espindula et al. (2018). The leaf aspect, crucial for photosynthesis, demonstrated a mean number of leaves close to that reported by Meneghelli et al. (2018), while mean leaf areas exceeded those reported by Santos et al. (2019). These findings suggest that, if these seedlings were planted in the field, they would exhibit higher vigor rates, indicating a correlation between the data obtained in this study and real-world conditions outside the laboratory.

The Dickson's quality index surpassed the threshold established by Hunt (1990) for obtaining quality seedlings (0.20), indicating a robust seedling vigor. Normal data distribution was observed for the traits shoot height, collar diameter and leaf area. According to Bussab & Morettin (2012), even when

the basic population is not normal, the distribution of sample means approaches normality for samples comprising more than 30 observations, validating the normal distribution assumption for all traits, given the 108 observations.

Considering the quality of seedlings from the blank trial, it is suggested that the data obtained from the evaluation of these seedlings are suitable for determining optimal plot sizes.

The coefficient of variation (CV) reflects data variability, with the number of leaves showing the highest CV among non-destructive traits, and the root dry matter exhibiting the highest CV among destructive traits, being consistent with the findings by Santos et al. (2019).

Table 3 presents the plot sizes for number of leaves per seedling focusing solely on non-destructive traits. For instance, if researchers intend to

Table 3. Optimal plot sizes (X₀) expressed as number of plants for evaluating the non-destructive trait number of leaves, estimated by the Hatheway's method in a randomized block design, in scenarios formed by combinations of number of treatments (I) with number of replications (J) in conilon coffee seedlings (*Coffea canephora* Pierre ex A. Froehner), clone LB1, with mean errors of 10, 20, 30 and 40 %, evaluated at 90 days after staking.

I	J	d = 10 %	d = 20 %	d = 30 %	d = 40 %	I	J	d = 10 %	d = 20 %	d = 30 %	d = 40 %
3	7	23	4	2	1	15	2	66	11	4	2
4	5	35	6	3	1	15	3	60	10	4	2
4	6	27	5	2	1	15	4	40	7	3	2
4	7	21	4	2	1	15	5	30	5	2	1
5	4	47	8	3	2	15	6	23	4	2	1
5	5	33	6	2	1	15	7	19	4	2	1
5	6	26	5	2	1	20	2	62	11	4	2
5	7	21	4	2	1	20	3	58	10	4	2
6	4	45	8	3	2	20	4	39	7	3	2
6	5	32	6	2	1	20	5	29	5	2	1
6	6	25	5	2	1	20	6	23	4	2	1
6	7	20	4	2	1	20	7	19	4	2	1
7	3	68	12	4	2	25	2	61	11	4	2
7	4	44	8	3	2	25	3	57	10	4	2
7	5	32	6	2	1	25	4	39	7	3	2
7	6	25	5	2	1	25	5	29	5	2	1
7	7	20	4	2	1	25	6	23	4	2	1
8	3	66	11	4	2	25	7	19	4	2	1
8	4	43	8	3	2	30	2	59	10	4	2
8	5	31	6	2	1	30	3	57	10	4	2
8	6	24	4	2	1	30	4	39	7	3	2
8	7	20	4	2	1	30	5	29	5	2	1
9	3	64	11	4	2	30	6	23	4	2	1
9	4	42	7	3	2	30	7	19	4	2	1
9	5	31	6	2	1	35	2	59	10	4	2
9	6	24	4	2	1	35	3	57	10	4	2
9	7	20	4	2	1	35	4	39	7	3	2
10	2	73	13	5	3	35	5	29	5	2	1
10	3	63	11	4	2	35	6	23	4	2	1
10	4	42	7	3	2	35	7	19	4	2	1
10	5	31	6	2	1	40	2	58	10	4	2
10	6	24	4	2	1	40	3	56	10	4	2
10	7	20	4	2	1	40	4	39	7	3	2

establish an experiment with LB1 seedlings in tubes to evaluate non-destructive traits, they could consider 6 treatments, 4 replications and 8 plants plot⁻¹, aiming at a precision mean error of 20 %, needing a total of 192 seedlings (6 x 4 x 8 = 192). Alternatively, with a lower accuracy requirement, such as a mean error of 30 %, they could opt for 6 treatments, 4 replications and 3 plants plot⁻¹, totaling 72 seedlings (6 x 4 x 3 = 72).

Table 4 is tailored for the destructive trait root dry matter, depicting plot sizes for scenarios involving different treatments, replications and experimental accuracies. For instance, if an experiment with a 20 % precision of the mean comprises 6 treatments, 4 replications and 42 plants plot⁻¹, a total of 1,008

seedlings would be necessary (6 x 4 x 42 = 1,008). Conversely, with a precision mean error of 30 %, the plot size should be 13 plants, requiring 312 seedlings (6 x 4 x 13 = 312).

As shown in Tables 3 and 4, d represents the experimental precision, with lower percentages indicating a higher accuracy and vice versa. This inverse relationship underscores that smaller differences between treatment means are deemed significant (Cargnelutti Filho et al. 2014). Consequently, smaller values for d lead to larger optimal plot sizes, given the same traits, number of treatments and replications (Celanti et al. 2016). Notably, the precision decreases as fewer plants per plot are used.

Table 4. Optimal plot sizes (X₀) expressed as number of plants for evaluation of the destructive trait root dry mass, estimated by the Hatheway's method in a randomized block design, in scenarios formed by combinations of number of treatments (I) with number of replications (J) in conilon coffee seedlings (*Coffea canephora* Pierre ex A. Froehner), clone LB1, with mean errors of 10, 20, 30 and 40 %, evaluated at 90 days after staking.

I	J	d = 10 %	d = 20 %	d = 30 %	d = 40 %	I	J	d = 10 %	d = 20 %	d = 30 %	d = 40 %
3	7	153	20	6	3	15	2	525	66	20	9
4	5	254	32	10	4	15	3	470	59	18	8
4	6	183	23	7	3	15	4	294	37	11	5
4	7	140	18	6	3	15	5	207	26	8	4
5	4	354	45	14	6	15	6	156	20	6	3
5	5	237	30	9	4	15	7	123	16	5	2
5	6	174	22	7	3	20	2	495	62	19	8
5	7	135	17	5	3	20	3	457	58	17	8
6	4	336	42	13	6	20	4	289	37	11	5
6	5	228	29	9	4	20	5	204	26	8	4
6	6	169	22	7	3	20	6	154	20	6	3
6	7	131	17	5	3	20	7	122	16	5	2
7	3	545	69	21	9	25	2	478	60	18	8
7	4	325	41	13	6	25	3	449	57	17	8
7	5	223	28	9	4	25	4	286	36	11	5
7	6	165	21	7	3	25	5	203	26	8	4
7	7	129	17	5	3	25	6	153	20	6	3
8	3	525	66	20	9	25	7	121	16	5	2
8	4	317	40	12	5	30	2	468	59	18	8
8	5	219	28	9	4	30	3	444	56	17	7
8	6	163	21	7	3	30	4	284	36	11	5
8	7	127	16	5	2	30	5	202	26	8	4
9	3	510	64	19	8	30	6	153	20	6	3
9	4	311	39	12	5	30	7	121	16	5	2
9	5	216	27	8	4	35	2	461	58	18	8
9	6	161	21	6	3	35	3	441	56	17	7
9	7	126	16	5	2	35	4	282	36	11	5
10	2	597	75	23	10	35	5	201	26	8	4
10	3	499	63	19	8	35	6	152	19	6	3
10	4	307	39	12	5	35	7	121	16	5	2
10	5	213	27	8	4	40	2	456	57	17	8
10	6	160	20	6	3	40	3	438	55	17	7
10	7	125	16	5	2	40	4	281	36	11	5

Achieving a high accuracy demands significant labor, financial resources and time, due to the requirement for many plants to constitute the plot size. In experiments with seedlings, the assessment necessitates sufficient plot sizes with a minimal number of seedlings per plot (Silva et al. 2010, Espindula et al. 2018). Opting for smaller plots and multiple replications enhances the efficiency of the experimental area utilization (Storck et al. 2011, Cargnelutti Filho et al. 2014). Determining the ideal plot size is crucial to accurately assess treatments and uphold result reliability (Cargnelutti Filho et al. 2021).

Maintaining the number of treatments while increasing the number of replications results in a greater number of plot sizes and a reduction in the number of seedlings required, being particularly evident in non-destructive traits (Table 3). Hence, using 4, 5, 6 or more replications can be advantageous when the experiment comprises 6 treatments. For instance, maintaining 6 treatments and increasing replications from 4 to 7 results in a reduction of 24 seedlings, thereby minimizing costs and labor, being consistent with findings by Schmildt et al. (2018) evaluating papaya plants in the field.

In short, whether more or fewer seedlings are used to constitute the plot is inconsequential. However, for a greater experimental accuracy (lower d%), more resources are required for seedlings, while higher d% necessitates fewer seedlings. Table 4, which addresses destructive traits, exemplifies this relationship. For instance, with a mean error of 10 %, an experiment comprising 8 treatments and 5 replications would require 219 plants plot⁻¹. Conversely, with a precision of 20 % around the mean, only 28 plants plot⁻¹ would be needed, with higher percentages leading to a decreased experimental accuracy.

CONCLUSION

Convenient plot size recommendations were obtained for clone LB1 conilon coffee seedlings grown in tubes, being observed that evaluating destructive traits necessitates larger plot sizes, when compared to non-destructive traits.

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