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Experimental dimensions and precision in trials with millet and slender leaf rattlebox[1]

Dimensionamentos experimentais e a precisão em ensaios com milheto e crotalária ochroleuca

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ABSTRACT - The objective of this study was to determine the optimal plot size to evaluate fresh matter in millet (*Pennisetum glaucum* L.) and slender leaf rattlebox (*Crotalaria ochroleuca*), in scenarios formed by combinations of numbers of treatments, numbers of replicates, and levels of precision. Fifteen uniformity trials with millet and slender leaf rattlebox, in single cropping or intercropping, were carried out. Fresh matter was evaluated in 540 basic experimental units (BEU) of 1 m × 1 m (15 trials × 36 BEU per trial). The soil heterogeneity index of Smith (1938) was estimated. Plot size was determined by the method of Hatheway (1961) in scenarios formed by combinations of i treatments (i = 5, 10, 15 and 20), r replicates (r = 3, 4, 5, 6, 7 and 8), and d precision levels (d = 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20%). To evaluate the fresh matter of millet and slender leaf rattlebox, in single or intercropping, in experiments in completely randomized or randomized block designs, with 5 to 20 treatments and with five replicates, plots with 10 m² of usable area are sufficient for differences between treatments of 10% of the overall mean of the experiment to be considered significant at 0.05 probability level.

Key words: *Pennisetum glaucum* L. *Crotalaria ochroleuca*. Soil cover crop. Uniformity trial. Optimal plot size.

RESUMO - O objetivo deste trabalho foi determinar o tamanho ótimo de parcela para avaliar a massa de matéria fresca de milheto (*Pennisetum glaucum* L.) e de crotalária ochroleuca (*Crotalaria ochroleuca*) em cenários formados por combinações de números de tratamentos, números de repetições e níveis de precisão experimental. Foram conduzidos 15 ensaios de uniformidade com milheto e crotalária ochroleuca, em cultivo solteiro e em consórcio. Foi avaliada a massa de matéria fresca em 540 unidades experimentais básicas (UEB) de 1 m × 1 m (15 ensaios × 36 UEB por ensaio). Foi estimado o índice de heterogeneidade do solo de Smith (1938). Foi determinado o tamanho de parcela por meio do método de Hatheway (1961) em cenários formados pelas combinações de i tratamentos (i = 5, 10, 15 e 20), r repetições (r = 3, 4, 5, 6, 7 e 8) e d níveis de precisão (d = 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% e 20%). Para avaliar a massa de matéria fresca de milheto e de crotalária ochroleuca, em cultivo solteiro ou em consórcio, nos delineamentos inteiramente casualizado ou de blocos completos ao acaso, com 5 a 20 tratamentos e com cinco repetições, parcelas de 10 m² de área útil são suficientes para que diferenças entre tratamentos de 10% da média geral do experimento sejam consideradas significativas a 0,05 de probabilidade.

Palavras-chave: *Pennisetum glaucum* L. *Crotalaria ochroleuca*. Cultura de cobertura de solo. Ensaio de uniformidade. Tamanho ótimo de parcela.

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INTRODUCTION

Soil cover species, such as millet (*Pennisetum glaucum* L.) and slender leaf rattlebox (*Crotalaria ochroleuca*), have been studied in relation to soil cover rate, decomposition rate, nutrient content and phytomass production (FERREIRA *et al*., 2019; PASSOS *et al*., 2017; PFÜLLER *et al*., 2019; VUICIK *et al*., 2018). In addition, the effects on the chemical and physical properties of the soil (ASCARI *et al*., 2020; NASCENTE; STONE, 2018; PASSOS *et al*., 2017; SOUSA *et al*., 2017; VUICIK *et al*., 2018), nematodes in soybean (DEBIASI *et al*., 2016), invasive plants (VUICIK *et al*., 2018) and, consequently, on the grain yields of rice, soybean and corn (ASCARI *et al*., 2020; DEBIASI *et al*., 2016; NASCENTE; STONE, 2018), have been investigated. These studies have pointed out beneficial aspects of these species in single cropping and in intercropping.

These studies were conducted with three replicates and plots of 24 m^2 (FERREIRA *et al*., 2019), four replicates and plots of 12 m^2 (PFÜLLER *et al*., 2019); 25 m^2 (ASCARI *et al*., 2020); 50 m^2 (PASSOS *et al*., 2017); 60 m^2 (DEBIASI *et al*., 2016); and 150 m^2 (SOUSA *et al*., 2017), five replicates and plots of 18 m^2 (VUICIK *et al*., 2018) and six replicates and plots of 168 m^2 (NASCENTE; STONE, 2018). In these studies, the criteria used to define the plot size and the number of replicates were not mentioned.

The application of the methodologies of Smith (1938) and Hatheway (1961) in a set of uniformity trials conducted with millet and slender leaf rattlebox, in single cropping or intercropping, makes it possible to calculate the optimal plot size according to the experimental design, number of treatments, number of replicates and experimental precision. These methodologies have been used in common bean (MAYOR-DURÁN; BLAIR; MUÑOZ, 2012), in sunflower (SOUSA *et al*., 2015; SOUSA; SILVA; ASSIS, 2016), banana (DONATO *et al*., 2018), cactus pear (GUIMARÃES *et al*., 2019, 2020) and in species with potential for soil cover, such as: turnip (CARGNELUTTI FILHO *et al*., 2014a); velvet bean (CARGNELUTTI FILHO *et al*., 2014b); flax (CARGNELUTTI FILHO *et al*., 2018) and black oats with common vetch (CARGNELUTTI FILHO *et al*., 2020).

Plot size has been investigated in millet (*Pennisetum glaucum* L.), cv. 'Comum' (BURIN *et al*., 2015, 2016) and in sunn hemp (*Crotalaria juncea* L.) (FACCO *et al*., 2017) through the maximum curvature of the coefficient of variation model (PARANAÍBA; FERREIRA; MORAIS, 2009) and also in *C. juncea* (FACCO *et al*., 2018) through the modified maximum curvature method (MEIER; LESSMAN, 1971). It is assumed that the intercropping, commonly used with soil cover plants, can generate distinct experimental planning patterns and, furthermore, that the use of the methodologies of Smith (1938) and Hatheway (1961), in another millet cultivar and in another sunn hemp species, can aggregate important information for the planning of experiments with these two soil cover plants.

Thus, the objective of this study was to determine the optimal plot size to evaluate the fresh matter of millet (*Pennisetum glaucum* L.) and slender leaf rattlebox (*Crotalaria ochroleuca*) in scenarios formed by combinations of numbers of treatments, numbers of replicates and levels of experimental precision.

MATERIAL AND METHODS

Fifteen uniformity trials were conducted with millet (*Pennisetum glaucum* L.), cultivar BRS 1501 (M), and slender leaf rattlebox (*Crotalaria ochroleuca*), cultivar 'Comum' (SLR), in an experimental area located at 29º42'S and 53º49'W at 95 m altitude. In this place, the climate is humid sub-tropical, Cfa, according to Köppen's classification, with hot summers and no dry season (ALVARES *et al*., 2013) and the soil is *Argissolo Vermelho Distrófico Arênico* (Ultisol) (SANTOS *et al*., 2018). The physical and chemical analysis of the soil at the 0-20 cm depth revealed: pH water 1:1: 5.2; Ca: 4.8 $cmol_c\ dm^{-3}$; Mg: 1.5 $cmol_c\ dm^{-3}$; Al: 0.3 $cmol_c\ dm^{-3}$; H+Al: 8.7 $cmol_c\ dm^{-3}$; SMP index: 5.4; organic matter: 2.3%; clay content: 24.0%; S: 15.3 mg dm^{-3}; P (Mehlich): 43.9 mg dm^{-3}; K: 0.593 $cmol_c\ dm^{-3}$; CEC_{pH7}: 15.6 $cmol_c\ dm^{-3}$; Cu: 1.77 mg dm^{-3}; Zn: 1.04 mg dm^{-3}; and B: 0.3 mg dm^{-3}.

Three uniformity trials (replicates) were conducted for each of the following five compositions, with the respective sowing densities in parentheses: 100% M (25 kg ha^{-1}); 75% M (18.75 kg ha^{-1}) + 25% SLR (4.6875 kg ha^{-1}): 50% M (12.5 kg ha^{-1}) + 50% SLR (9.375 kg ha^{-1}); 25% M (6.25 kg ha^{-1}) + 75% SLR (14.0625 kg ha^{-1}); and 100% SLR (18.75 kg ha^{-1}). Therefore, in total, 15 uniformity trials were conducted (3 trials/composition × 5 compositions = 15 trials). On November 13, 2019, basal fertilization was performed with 20 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5 and 80 kg ha^{-1} of K_2O (N-P-K, formulation 05-20-20), followed by broadcast sowing. On December 18, 2019, 40 kg ha^{-1} of N was applied in the form of urea.

In each uniformity trial, the central area with size of 6 m × 6 m (36 m^2) was divided into 36 basic experimental units (BEU) of 1 m × 1 m (1 m^2) forming a matrix of six rows and six columns. On January 29 and 30, 2020, in the flowering of millet, in each BEU, the plants were cut near the soil surface and their fresh matter (FM) was immediately weighed, expressed in g m^{-2}. Weighing was performed immediately after cutting in order to minimize possible variations in plant moisture.

For each uniformity trial, from the FM data of the 36 BEU, plots with X_R BEU adjacent in the row and X_C BEU adjacent in the column were planned. The plots with different sizes and/or shapes were planned as being ($X=X_R \times X_C$), that is, (1×1), (1×2), (1×3), (1×6), (2×1), (2×2), (2×3), (2×6), (3×1), (3×2), (3×3), (3×6), (6×1), (6×2) and (6×3). The acronyms X_R, X_C and X respectively mean number of BEU adjacent in the row, number of BEU adjacent in the column, and plot size in number of BEU.

For each plot size (X), the following parameters were determined: n - number of plots with X BEU in size (n=36/X); $M_{(X)}$ - mean of plots with X BEU in size; $V_{(X)}$ - variance between plots with X BEU in size; $CV_{(X)}$ - coefficient of variation (in %) between the plots with X BEU in size; and $VU_{(X)}$ - variance per BEU between the plots with X BEU in size [$VU_{(X)}=V_{(X)}/X^2$].

The parameters V1 (estimate of variance per BEU between the plots with size of one BEU) and b (estimate of soil heterogeneity index) and the coefficient of determination (r^2) of the function $VU_{(X)}=V1/X^b$, of Smith (1938), were estimated. These parameters were estimated by logarithmic transformation and linearization of the function $VU_{(X)}=V1/X^b$, that is, $\log VU_{(X)} = \log V1 - b \log X$, whose estimation was weighted by the degrees of freedom (DF=n-1), associated with each plot size, according to the application of Sousa, Silva and Assis (2016). The observed values of dependent variables [$VU_{(X)}$] and independent variables (X) and the function $VU_{(X)}=V1/X^b$ (SMITH, 1938) were plotted.

Experimental plans were simulated in the completely randomized and randomized complete block design for the scenarios formed by the combinations of i treatments (i = 5, 10, 15 and 20), r replicates (r = 3, 4, 5, 6, 7 and 8) and d differences between treatment means to be detected as significant at 0.05 probability level, expressed as a percentage of the overall mean of the experiment, that is, in precision levels [d = 2% (higher precision), 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20% (lower precision)].

For each experimental plan, the optimal plot size (Xo), in number of BEU (approximated to the next integer), was calculated using the expression $Xo = \sqrt[b]{2\,(t_1+t_2)^2 CV^2/rd^2}$ (HATHEWAY, 1961). In this expression, b is the estimate of the soil heterogeneity index (in this study, for each composition, the mean of b of the three uniformity trials was considered); t_1 is the critical value of Student's t-distribution for the significance level of the test (type I error) of α=5% (bilateral test at 5%), with DF degrees of freedom; t_2 is the critical value of Student's t-distribution, corresponding to 2(1-P) (bilateral test), where P is the probability of obtaining significant results, that is, the power of the test (P=0.80, in this study), with DF degrees of freedom; CV is the estimate of the coefficient of variation between the plots with size of one BEU (in this study, for each composition, the mean of CV of the three uniformity trials was considered), in percentage; r is the number of replicates and d is the difference between treatment means to be detected as significant at 0.05 probability level, expressed as a percentage of the overall mean of the experiment (precision). The degrees of freedom (DF) to obtain the critical values (tabulate) of the Student's t-distribution were obtained by the expressions DF=(i)(r-1), for the completely randomized design, and DF=(i-1)(r-1), for the randomized complete block design, where i is the number of treatments and r is the number of replicates. The values of t_1 and t_2 in this study were obtained with the Microsoft Office Excel® application, using the functions t_1=INVT(0.05;DF) and t_2=INVT(0.40;DF), respectively. Statistical analyses were performed with Microsoft Office Excel®.

RESULTS AND DISCUSSION

In the 15 uniformity trials, formed by compositions of sowing densities of millet (*Pennisetum glaucum* L.), cultivar BRS 1501 (M) and slender leaf rattlebox (*Crotalaria ochroleuca*), cultivar 'Comum' (SLR), the fresh matter (FM) fluctuated between 4413 and 9077 g m^{-2}, that is, 44.13 and 90.77 Mg ha^{-1}, respectively (Table 1). The FM means of the three trials of each composition were 7325, 7812, 8466, 8505 and 4511 g m^{-2}, for the compositions of 100% M, 75% M + 25% SLR, 50% M + 50% SLR, 25% M + 75% SLR and 100% SLR, respectively. The FM mean of the three compositions in intercropping - 75% M + 25% SLR, 50% M + 50% SLR, 25% M + 75% SLR - (8261 g m^{-2}) was higher than the mean of the single crops of millet - 100% M - (7325 g m^{-2}; t = 2.882; p-value = 0.016318; with 10 degrees of freedom) and slender leaf rattlebox - 100% SLR - (4511 g m^{-2}; t = 11.653; p-value < 0.0000001; with 10 degrees of freedom). Among single crops, the FM of millet was higher than that of slender leaf rattlebox (t = 20.39212; p-value = 0.000034; with 4 degrees of freedom). For these same cultivars of millet and slender leaf rattlebox, Passos *et al*. (2017) obtained FM of 34.59 Mg ha^{-1} and 31.35 Mg ha^{-1} and Pfüller *et al*. (2019) obtained 5.327 and 2.536 Mg ha^{-1}, respectively.

The coefficient of variation (CV) of FM, obtained from the 36 BEU of each of the 15 uniformity trials, ranged from 10.93% to 17.84% (Table 1). The means of CV of the three trials of each composition were 12.58%, 12.24%, 15.05%, 15.72% and 14.74%, for the compositions of 100% M, 75% M + 25% SLR, 50% M + 50% SLR, 25% M + 75% SLR and 100% SLR, respectively. All coefficients, with these magnitudes, are considered medium according to Pimentel-Gomes classification (2009) for agricultural crops in general, that is, they are within the range from 10% to 20%. This suggests that experiments with millet and slender leaf rattlebox, in single cropping or intercropping, have similar experimental precision. CV variations between compositions may be associated with environmental variability, genotypic variability and interaction of the genotype with the environment.

Table 1 - Planned plot size ($X=X_R \times X_C$), in basic experimental units (BEU), with X_R BEU adjacent in row and X_C BEU adjacent in column; number of plots with X BEU in size (n=36/X); mean of plots with X BEU in size [$M_{(X)}$], in g; and coefficient of variation (in %) between the plots with X BEU in size [$CV_{(X)}$]. Fresh matter data for sowing densities of millet (M) and slender leaf rattlebox (SLR)

T[(1)]	X_R	X_C	X	n	100% M		75% M + 25% SLR		50% M + 50% SLR		25% M + 75% SLR		100% SLR	
					$M_{(X)}$	$CV_{(X)}$	$M_{(X)}$	$CV_{(X)}$	$M_{(X)}$	$CV_{(X)}$	$M_{(X)}$	$CV_{(X)}$	$M_{(X)}$	$CV_{(X)}$
1	1	1	1	36	7299	12.14	7349	11.08	8747	14.85	7843	15.44	4453	17.67
1	1	2	2	18	14598	9.38	14698	7.64	17494	12.51	15686	12.38	8906	11.85
1	1	3	3	12	21897	6.23	22047	6.54	26240	11.28	23529	10.58	13359	10.95
1	1	6	6	6	43793	1.98	44095	5.57	52481	8.14	47058	9.29	26719	7.60
1	2	1	2	18	14598	10.17	14698	7.15	17494	10.10	15686	10.84	8906	13.51
1	2	2	4	9	29195	8.10	29396	4.33	34987	8.54	31372	9.93	17812	8.24
1	2	3	6	6	43793	4.91	44095	2.30	52481	6.10	47058	9.02	26719	8.63
1	2	6	12	3	87586	1.50	88189	0.23	104961	2.81	94116	9.00	53437	5.74
1	3	1	3	12	21897	7.62	22047	5.88	26240	9.19	23529	10.25	13359	12.27
1	3	2	6	6	43793	6.29	44095	3.67	52481	8.65	47058	8.55	26719	7.05
1	3	3	9	4	65690	4.77	66142	1.08	78721	7.53	70587	8.37	40078	7.37
1	3	6	18	2	131380	0.61	132284	0.02	157442	5.60	141174	9.27	80156	3.88
1	6	1	6	6	43793	7.48	44095	5.72	52481	8.21	47058	6.06	26719	9.49
1	6	2	12	3	87586	6.84	88189	3.90	104961	8.17	94116	4.97	53437	3.52
1	6	3	18	2	131380	5.69	132284	0.40	157442	7.08	141174	4.37	80156	8.11
2	1	1	1	36	7142	10.93	8042	12.75	8618	13.78	8594	13.89	4665	12.18
2	1	2	2	18	14285	8.18	16084	6.25	17236	9.98	17188	9.83	9330	7.49
2	1	3	3	12	21427	7.96	24126	3.83	25854	7.29	25782	6.40	13995	7.75
2	1	6	6	6	42855	5.12	48251	2.92	51708	5.67	51565	5.54	27990	3.65
2	2	1	2	18	14285	6.93	16084	10.99	17236	11.09	17188	9.69	9330	9.85
2	2	2	4	9	28570	5.21	32167	5.34	34472	8.41	34377	8.36	18660	4.97
2	2	3	6	6	42855	4.15	48251	1.92	51708	5.67	51565	5.19	27990	6.03
2	2	6	12	3	85709	1.68	96502	1.30	103415	3.78	103130	4.95	55981	3.08
2	3	1	3	12	21427	5.90	24126	8.75	25854	8.66	25782	7.74	13995	9.91
2	3	2	6	6	42855	3.93	48251	4.03	51708	7.63	51565	7.00	27990	5.59
2	3	3	9	4	64282	2.43	72377	2.93	77561	4.34	77347	4.07	41986	7.02
2	3	6	18	2	128564	1.84	144753	3.11	155123	5.27	154695	3.53	83971	2.86
2	6	1	6	6	42855	4.52	48251	5.34	51708	7.07	51565	5.53	27990	8.60
2	6	2	12	3	85709	1.43	96502	2.30	103415	6.95	103130	5.81	55981	1.22
2	6	3	18	2	128564	2.31	144753	0.85	155123	0.33	154695	3.33	83971	5.70
3	1	1	1	36	7534	14.68	8044	12.89	8034	16.52	9077	17.84	4413	14.38
3	1	2	2	18	15068	10.89	16088	9.79	16068	13.17	18155	13.17	8827	11.06
3	1	3	3	12	22602	8.81	24131	7.10	24102	11.52	27232	11.61	13240	9.63
3	1	6	6	6	45204	8.21	48263	5.18	48204	10.35	54465	10.68	26480	5.25
3	2	1	2	18	15068	11.71	16088	8.48	16068	11.06	18155	11.46	8827	8.54
3	2	2	4	9	30136	9.52	32175	7.15	32136	7.05	36310	8.04	17654	7.14
3	2	3	6	6	45204	8.47	48263	4.22	48204	6.30	54465	6.57	26480	4.81
3	2	6	12	3	90408	9.05	96526	2.28	96409	4.83	108929	4.33	52961	2.77

Continuation table 1

3	3	1	3	12	22602	8.39	24131	8.04	24102	9.17	27232	7.83	13240	4.67
3	3	2	6	6	45204	7.14	48263	7.48	48204	6.04	54465	5.45	26480	4.39
3	3	3	9	4	67806	5.68	72394	2.35	72307	5.65	81697	4.57	39721	1.49
3	3	6	18	2	135612	6.70	144789	1.57	144613	3.02	163394	4.82	79441	1.01
3	6	1	6	6	45204	5.53	48263	5.15	48204	6.05	54465	6.03	26480	2.31
3	6	2	12	3	90408	4.05	96526	5.22	96409	3.48	108929	2.09	52961	1.25
3	6	3	18	2	135612	1.37	144789	1.43	144613	4.08	163394	0.62	79441	1.20

(1) Each uniformity trial of size 6 m × 6 m (36 m^2) was divided into 36 BEU of 1 m × 1 m (1 m^2), forming an matrix of six rows and six columns

The soil heterogeneity index (b) of Smith (1938), among the 15 uniformity trials, ranged from 0.6587 to 1.7891 (Figure 1). The means of b of the three trials of each composition were 1.0330, 1.4709, 0.9183, 0.9535 and 1.1444 for the compositions of 100% M, 75% M + 25% SLR, 50% M + 50% SLR, 25% M + 75% SLR and 100% SLR, respectively. According to Smith (1938), the index value describes, in addition to soil heterogeneity, other variations, such as those related to plant production, climatic conditions, management and experimental data collection. The presence of these sources of variability tend to increase the value of the soil heterogeneity index (b). The values close to the unit indicate high soil heterogeneity or low correlation between adjacent plots. According to Lin and Binns (1986), when $b > 0.7$, plot size should be increased, when $b < 0.2$, the number of replicates should be increased and, in cases of $0.2 \leq b \leq 0.7$, the researcher should investigate the best combination between plot size and number of replicates. Therefore, the high values of b and the similarity between the compositions suggest that experiments with millet and slender leaf rattlebox in single cropping or intercropping, should place greater emphasis on the use of larger plots.

In the 15 uniformity trials, there were reductions in the coefficient of variation [$CV_{(X)}$] and in the variance per BEU between the plots [$VU_{(X)}$], with the increase in the planned plot size (X) (Table 1 and Figure 1). Then, it can be inferred that there is improvement in experimental precision (decrease in $CV_{(X)}$ and $VU_{(X)}$) with the increase in plot size. In practice, as demonstrated in this study, it is possible to evaluate the fresh matter (FM) in plots of 1 m^2. However, smaller plots may not represent the development of plants in single cropping and intercropping. Conversely, larger sizes would make it possible to evaluate the plants in the central area of the plot (usable area) and disregard the borders, thus reducing the interference of plants of the adjacent plots, that is, the inter-plot competition. Thus, it is important to determine the optimal plot size to ensure adequate discrimination of treatments under evaluation and reliability in the inferences.

There were marked reductions in variance per BEU [$VU_{(X)}$] with plots of up to four BEU in size (4 m^2), intermediate reductions with plots between four and ten BEU, and stabilization trend with plots larger than ten BEU (Figure 1). In species with potential for soil cover, such as: turnip (CARGNELUTTI FILHO *et al*., 2014a); velvet bean (CARGNELUTTI FILHO *et al*., 2014b); flax (CARGNELUTTI FILHO *et al*., 2018); and black oats with common vetch (CARGNELUTTI FILHO *et al*., 2020), the pattern was similar. Therefore, to evaluate the fresh matter of millet and slender leaf rattlebox, in single cropping or intercropping, a plot of up to ten BEU (10 m^2) is suggested because the gain in experimental precision (decrease in $VU_{(X)}$) with progressive increases in plot size, from ten BEU, was not significant. This value of 10 m^2 is relatively higher than the optimal plot size required to evaluate the fresh matter of millet, cv. 'Comum', which was 4.46 m^2 in three evaluation times (BURIN *et al*., 2015) and 4.97 m^2, for the three times of sowing and cuts (BURIN *et al*., 2016). It was also higher than the sizes of 2.04 m^2 (FACCO *et al*., 2017) and 1.98 m^2 (FACCO *et al*., 2018) to evaluate the fresh matter of sunn hemp. The differences between the environments, millet cultivars and sunn hemp species and also the methodologies used to determine plot size contribute to explaining the different results from those obtained in this study.

In the methodology of Hatheway (1961), based on fixed values of the soil heterogeneity index (b) of Smith (1938) and coefficient of variation (CV), it is possible to determine different optimal plot sizes (Xo), as a function of the number of treatments (i), number of replicates (r) and precision (d) (Tables 2 and 3). The results obtained using this methodology allow the researcher to investigate within his/her availability of experimental area, number of treatments to be evaluated and desired precision, which combination of plot size and number of replicates is more appropriate.

With fixed values of i and r, the Xo increased with the increment in precision (d) (Tables 2 and 3). For example, to evaluate FM in an experiment with millet in single cropping (100% M), conducted in a completely randomized design (CRD), with five treatments and three replicates, aiming that in 80% of the experiments

Figure 1 - Relationship between variance per basic experimental unit (BEU) between plots with X BEU in size ($[VU_{(X)}=V_{(X)}/X^2]$ and the planned plot size (X), in BEU, and estimates of parameters of the function $VU_{(X)}=V1/X^b$ of Smith (1938). Fresh matter data obtained in uniformity trials, with 36 BEU of 1 m², formed by compositions of sowing densities of millet (*Pennisetum glaucum* L.), cultivar BRS 1501 (M), and slender leaf rattlebox (*Crotalaria ochroleuca*), cultivar 'Comum' (SLR)

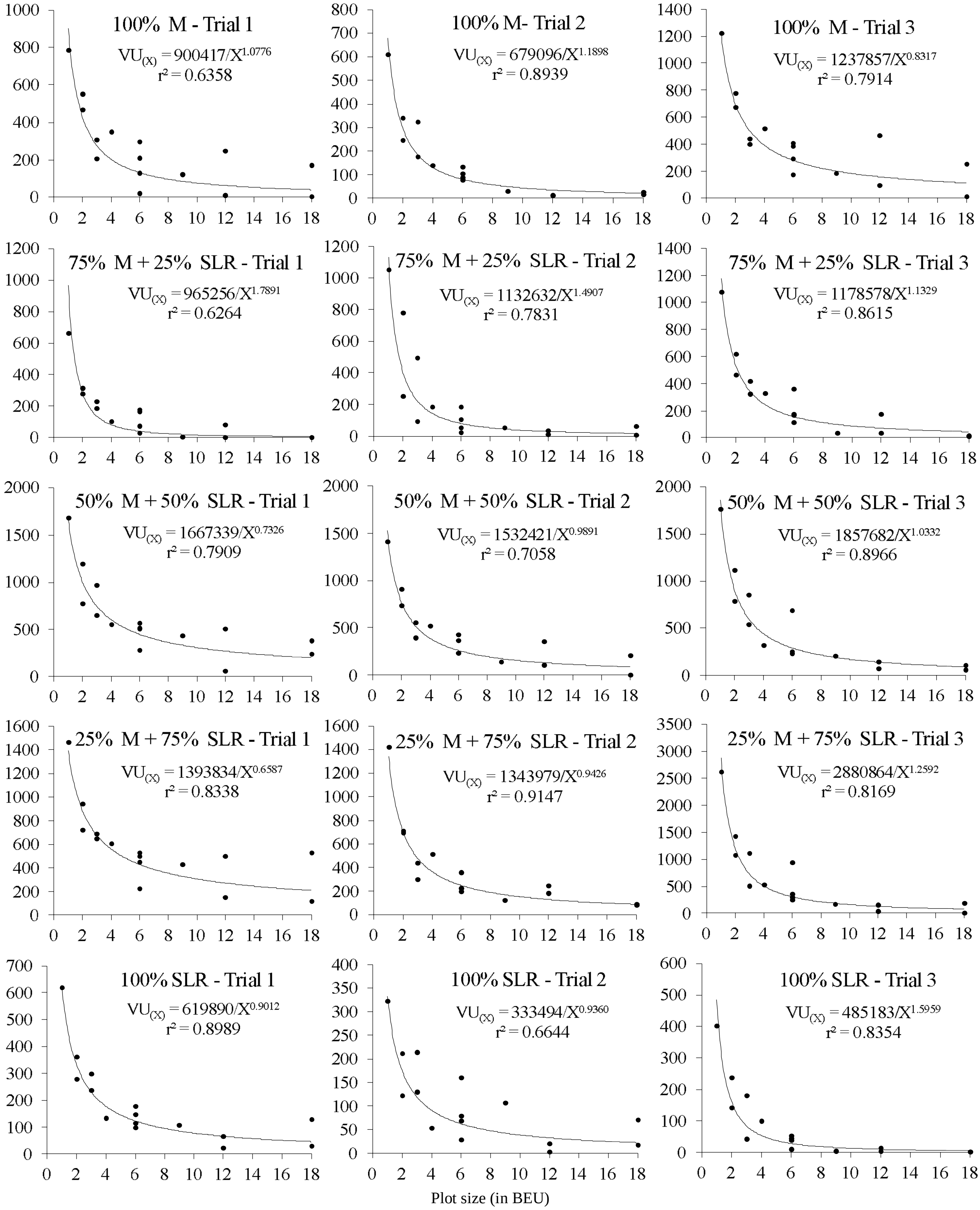

Table 2 - Optimal plot size, in m², for completely randomized design, in combinations of i treatments, r replicates and d precision levels, for fresh matter at sowing densities of millet and slender leaf rattlebox

d (%)	i = 5 treatments						i = 10 treatments						i = 15 treatments						i = 20 treatments					
	r=3	r=4	r=5	r=6	r=7	r=8	r=3	r=4	r=5	r=6	r=7	r=8	r=3	r=4	r=5	r=6	r=7	r=8	r=3	r=4	r=5	r=6	r=7	r=8
100% millet (soil heterogeneity index b =1.0330; CV = 12.58%)																								
2	214	151	118	97	82	72	193	141	112	93	80	70	187	138	110	92	79	69	184	137	110	91	79	69
4	56	40	31	26	22	19	51	37	30	25	21	19	49	37	29	24	21	18	48	36	29	24	21	18
6	26	18	14	12	10	9	23	17	14	12	10	9	23	17	14	11	10	9	22	17	13	11	10	9
8	15	11	9	7	6	5	14	10	8	7	6	5	13	10	8	7	6	5	13	10	8	7	6	5
10	10	7	6	5	4	4	9	7	5	5	4	4	9	7	5	5	4	4	9	7	5	5	4	4
12	7	5	4	4	3	3	6	5	4	3	3	3	6	5	4	3	3	3	6	5	4	3	3	3
14	5	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2
16	4	3	3	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2
18	4	3	2	2	2	2	3	3	2	2	2	1	3	2	2	2	2	1	3	2	2	2	2	1
20	3	2	2	2	1	1	3	2	2	2	1	1	3	2	2	2	1	1	3	2	2	2	1	1
75% millet + 25% slender leaf rattlebox (soil heterogeneity index b = 1.4709; CV = 12.24%)																								
2	42	33	28	24	22	20	39	32	27	24	21	19	38	31	27	23	21	19	38	31	26	23	21	19
4	17	13	11	10	9	8	16	13	11	10	9	8	15	12	11	9	9	8	15	12	11	9	9	8
6	10	8	7	6	5	5	9	7	6	6	5	5	9	7	6	6	5	5	9	7	6	6	5	5
8	7	5	5	4	4	3	6	5	5	4	4	3	6	5	4	4	4	3	6	5	4	4	4	3
10	5	4	4	3	3	3	5	4	3	3	3	3	5	4	3	3	3	3	5	4	3	3	3	3
12	4	3	3	3	2	2	4	3	3	3	2	2	4	3	3	3	2	2	4	3	3	3	2	2
14	3	3	2	2	2	2	3	3	2	2	2	2	3	3	2	2	2	2	3	3	2	2	2	2
16	3	2	2	2	2	2	3	2	2	2	2	2	3	2	2	2	2	2	3	2	2	2	2	2
18	3	2	2	2	2	1	2	2	2	2	2	1	2	2	2	2	2	1	2	2	2	2	2	1
20	2	2	2	2	1	1	2	2	2	2	1	1	2	2	2	2	1	1	2	2	2	1	1	1
50% millet + 50% slender leaf rattlebox (soil heterogeneity index b = 0.9183; CV = 15.05%)																								
2	616	417	315	253	211	180	549	387	298	242	203	175	529	378	293	238	201	173	519	373	290	237	199	172
4	137	93	70	56	47	40	122	86	66	54	45	39	117	84	65	53	45	39	115	83	64	53	44	38
6	57	39	29	24	20	17	51	36	28	23	19	16	49	35	27	22	19	16	48	35	27	22	19	16
8	31	21	16	13	11	9	27	19	15	12	10	9	26	19	15	12	10	9	26	19	15	12	10	9
10	19	13	10	8	7	6	17	12	9	8	7	6	16	12	9	8	7	6	16	12	9	8	6	6
12	13	9	7	6	5	4	12	8	7	5	5	4	11	8	6	5	5	4	11	8	6	5	5	4
14	9	7	5	4	4	3	8	6	5	4	3	3	8	6	5	4	3	3	8	6	5	4	3	3
16	7	5	4	3	3	2	6	5	4	3	3	2	6	5	4	3	3	2	6	5	4	3	3	2
18	6	4	3	3	2	2	5	4	3	3	2	2	5	4	3	2	2	2	5	4	3	2	2	2
20	5	3	3	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2
25% millet + 75% slender leaf rattlebox (soil heterogeneity index b = 0.9535; CV = 15.72%)																								
2	533	366	279	226	189	163	477	340	265	216	183	158	460	332	260	213	181	157	452	328	258	212	180	156
4	125	86	66	53	45	38	112	80	62	51	43	37	108	78	61	50	43	37	106	77	61	50	42	37
6	54	37	28	23	19	17	48	34	27	22	19	16	46	34	26	22	18	16	46	33	26	22	18	16
8	30	20	16	13	11	9	26	19	15	12	10	9	26	19	15	12	10	9	25	18	15	12	10	9
10	19	13	10	8	7	6	17	12	10	8	7	6	16	12	9	8	7	6	16	12	9	8	7	6
12	13	9	7	6	5	4	12	8	7	6	5	4	11	8	7	5	5	4	11	8	7	5	5	4
14	9	7	5	4	4	3	9	6	5	4	4	3	8	6	5	4	4	3	8	6	5	4	4	3
16	7	5	4	3	3	3	7	5	4	3	3	3	6	5	4	3	3	2	6	5	4	3	3	2
18	6	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2
20	5	3	3	2	2	2	4	3	3	2	2	2	4	3	3	2	2	2	4	3	3	2	2	2
100% slender leaf rattlebox (soil heterogeneity index b = 1.1444; CV = 14.74%)																								
2	167	122	98	82	71	63	153	115	94	79	69	61	148	113	92	78	68	61	146	112	92	78	68	60
4	50	37	29	25	21	19	46	35	28	24	21	19	44	34	28	24	21	18	44	34	28	24	21	18

Continuation table 2

6	25	18	15	12	11	10	23	17	14	12	11	9	22	17	14	12	10	9	22	17	14	12	10	9
8	15	11	9	8	7	6	14	11	9	7	7	6	14	10	9	7	7	6	13	10	9	7	6	6
10	11	8	6	5	5	4	10	7	6	5	5	4	9	7	6	5	5	4	9	7	6	5	5	4
12	8	6	5	4	4	3	7	6	5	4	3	3	7	5	5	4	3	3	7	5	4	4	3	3
14	6	5	4	3	3	3	6	4	4	3	3	3	5	4	4	3	3	3	5	4	4	3	3	2
16	5	4	3	3	2	2	5	4	3	3	2	2	4	3	3	3	2	2	4	3	3	3	2	2
18	4	3	3	2	2	2	4	3	3	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2
20	3	3	2	2	2	2	3	3	2	2	2	2	3	3	2	2	2	2	3	2	2	2	2	2

Table 3 - Optimal plot size, in m^2, for randomized complete block design, in combinations of i treatments, r replicates and d precision levels, for fresh matter at sowing densities of millet and slender leaf rattlebox

d (%)	i = 5 treatments						i = 10 treatments						i = 15 treatments						i = 20 treatments					
	r=3	r=4	r=5	r=6	r=7	r=8	r=3	r=4	r=5	r=6	r=7	r=8	r=3	r=4	r=5	r=6	r=7	r=8	r=3	r=4	r=5	r=6	r=7	r=8
100% millet (soil heterogeneity index b =1.0330; CV = 12.58%)																								
2	226	156	121	99	84	73	195	142	113	94	80	70	188	139	111	92	79	69	184	137	110	92	79	69
4	59	41	32	26	22	19	51	38	30	25	21	19	49	37	29	24	21	18	48	36	29	24	21	18
6	27	19	15	12	10	9	24	17	14	12	10	9	23	17	14	11	10	9	22	17	14	11	10	9
8	16	11	9	7	6	5	14	10	8	7	6	5	13	10	8	7	6	5	13	10	8	7	6	5
10	10	7	6	5	4	4	9	7	5	5	4	4	9	7	5	5	4	4	9	7	5	5	4	4
12	8	5	4	4	3	3	7	5	4	3	3	3	6	5	4	3	3	3	6	5	4	3	3	3
14	6	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2
16	5	3	3	2	2	2	4	3	3	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2
18	4	3	2	2	2	2	3	3	2	2	2	1	3	2	2	2	2	1	3	2	2	2	2	1
20	3	2	2	2	1	1	3	2	2	2	1	1	3	2	2	2	1	1	3	2	2	2	1	1
75% millet + 25% slender leaf rattlebox (soil heterogeneity index b = 1.4709; CV = 12.24%)																								
2	44	34	28	25	22	20	40	32	27	24	21	19	38	31	27	24	21	19	38	31	27	23	21	19
4	17	14	11	10	9	8	16	13	11	10	9	8	15	12	11	9	9	8	15	12	11	9	9	8
6	10	8	7	6	5	5	9	8	6	6	5	5	9	7	6	6	5	5	9	7	6	6	5	5
8	7	6	5	4	4	3	6	5	5	4	4	3	6	5	4	4	4	3	6	5	4	4	4	3
10	5	4	4	3	3	3	5	4	3	3	3	3	5	4	3	3	3	3	5	4	3	3	3	3
12	4	3	3	3	2	2	4	3	3	3	2	2	4	3	3	3	2	2	4	3	3	3	2	2
14	4	3	2	2	2	2	3	3	2	2	2	2	3	3	2	2	2	2	3	3	2	2	2	2
16	3	2	2	2	2	2	3	2	2	2	2	2	3	2	2	2	2	2	3	2	2	2	2	2
18	3	2	2	2	2	1	2	2	2	2	2	1	2	2	2	2	2	1	2	2	2	2	2	1
20	2	2	2	2	1	1	2	2	2	2	1	1	2	2	2	2	1	1	2	2	2	2	1	1
50% millet + 50% slender leaf rattlebox (soil heterogeneity index b = 0.9183; CV = 15.05%)																								
2	655	433	324	258	214	183	556	390	300	243	204	175	532	379	293	239	201	173	521	374	290	237	200	172
4	145	96	72	57	48	41	123	87	67	54	45	39	118	84	65	53	45	39	115	83	65	53	44	38
6	60	40	30	24	20	17	51	36	28	23	19	16	49	35	27	22	19	16	48	35	27	22	19	16
8	32	22	16	13	11	9	28	20	15	12	10	9	26	19	15	12	10	9	26	19	15	12	10	9
10	20	14	10	8	7	6	17	12	9	8	7	6	16	12	9	8	7	6	16	12	9	8	6	6
12	14	9	7	6	5	4	12	8	7	5	5	4	11	8	6	5	5	4	11	8	6	5	5	4
14	10	7	5	4	4	3	9	6	5	4	3	3	8	6	5	4	3	3	8	6	5	4	3	3
16	8	5	4	3	3	2	6	5	4	3	3	2	6	5	4	3	3	2	6	5	4	3	3	2
18	6	4	3	3	2	2	5	4	3	3	2	2	5	4	3	2	2	2	5	4	3	2	2	2
20	5	3	3	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2
25% millet + 75% slender leaf rattlebox (soil heterogeneity index b = 0.9535; CV = 15.72%)																								
2	565	380	287	231	193	166	482	343	266	217	184	159	462	333	261	214	181	157	453	329	258	212	180	156
4	132	89	67	54	45	39	113	81	63	51	43	37	108	78	61	50	43	37	106	77	61	50	42	37

Continuation table 3

6	57	38	29	23	20	17	49	35	27	22	19	16	47	34	26	22	19	16	46	33	26	22	18	16
8	31	21	16	13	11	10	27	19	15	12	10	9	26	19	15	12	10	9	25	18	15	12	10	9
10	20	13	10	8	7	6	17	12	10	8	7	6	16	12	9	8	7	6	16	12	9	8	7	6
12	14	9	7	6	5	4	12	8	7	6	5	4	11	8	7	5	5	4	11	8	7	5	5	4
14	10	7	5	4	4	3	9	6	5	4	4	3	8	6	5	4	4	3	8	6	5	4	4	3
16	8	5	4	3	3	3	7	5	4	3	3	3	6	5	4	3	3	2	6	5	4	3	3	2
18	6	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2	5	4	3	3	2	2
20	5	4	3	2	2	2	4	3	3	2	2	2	4	3	3	2	2	2	4	3	3	2	2	2
100% slender leaf rattlebox (soil heterogeneity index b = 1.1444; CV = 14.74%)																								
2	176	126	100	84	72	63	154	116	94	80	69	61	149	113	92	78	68	61	146	112	92	78	68	60
4	53	38	30	25	22	19	46	35	28	24	21	19	45	34	28	24	21	18	44	34	28	24	21	18
6	26	19	15	13	11	10	23	17	14	12	11	9	22	17	14	12	10	9	22	17	14	12	10	9
8	16	12	9	8	7	6	14	11	9	8	7	6	14	11	9	7	7	6	13	10	9	7	6	6
10	11	8	6	5	5	4	10	7	6	5	5	4	9	7	6	5	5	4	9	7	6	5	5	4
12	8	6	5	4	4	3	7	6	5	4	3	3	7	5	5	4	3	3	7	5	4	4	3	3
14	6	5	4	3	3	3	6	4	4	3	3	3	5	4	4	3	3	3	5	4	4	3	3	2
16	5	4	3	3	2	2	5	4	3	3	2	2	4	3	3	3	2	2	4	3	3	3	2	2
18	4	3	3	2	2	2	4	3	3	2	2	2	4	3	2	2	2	2	4	3	2	2	2	2
20	4	3	2	2	2	2	3	3	2	2	2	2	3	3	2	2	2	2	3	2	2	2	2	2

(power=0.80) differences between treatments of d=20% of the overall mean of the experiment (lower precision) are detected as significant at 5% probability level, the plot size should be three BEU (3 m^2) (Table 2). Plots of 10 m^2 would make it possible to improve precision, that is, to obtain d=10%. To further increase precision, that is, to obtain d=2%, a plot with 214 BEU (214 m^2) would be necessary. Obviously, the experimental precision is higher, but conducting field experiment with a plot of 214 m^2 is impractical. Therefore, high experimental precisions (low percentages of d) are difficult to be achieved in practice, due to the need for large plot size, as already pointed out by Cargnelutti Filho *et al*. (2014a, 2014b, 2018, 2020). A similar pattern was observed in the compositions of 75% M + 25% SLR, 50% M + 50% SLR, 25% M + 75% SLR and 100% SLR (Tables 2 and 3).

With fixed values of i and d, the Xo decreased with the increment in r. Also, with fixed values of r and d, there was a reduction in Xo with the increase in i (Tables 2 and 3). The higher the number of treatments and the number of replicates, the greater the number of degrees of freedom of the error and, consequently, the lower the estimate of the residual variance (mean square of the error), that is, the greater the experimental precision.

The information provided in this study allows investigations in 240 scenarios formed by combinations of i treatments (i = 5, 10, 15 and 20), r replicates (r = 3, 4, 5, 6, 7 and 8) and d differences between treatment means to be detected as significant at 5% probability level (d = 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20%), for each composition and each experimental design (Tables 2 and 3). For example, if the researcher wants to evaluate the FM of five treatments of the composition formed by 50% millet + 50% slender leaf rattlebox (50% M + 50% SLR), in the CRD, and wants a precision (d) of 10%, among the various options, he/she can use plots of 19 BEU (19 m^2) and three replicates, 13 BEU (13 m^2) and four replicates, ten BEU (10 m^2) and five replicates, eight BEU (8 m^2) and six replicates, seven BEU (7 m^2) and seven replicates or six BEU (6 m^2) and eight replicates (Table 2). In this same scenario, in the randomized complete block design (RCBD), he/she could use plots of 20, 14, 10, 8, 7, 6 m^2, with 3, 4, 5, 6, 7 and 8 replicates, respectively (Table 3). For fixed values of i, r and d, the composition 50% M + 50% SLR, with soil heterogeneity index b = 0.9183 and CV = 15.05%, had the largest plot sizes, compared to the other compositions (100% M, 75% M + 25% SLR, 25% M + 75% SLR and 100% SLR), in both designs (Tables 2 and 3). Thus, the results of this composition can be used as a reference for the definition of plot size and number of replicates to ensure sufficient experimental precision in experiments with millet and slender leaf rattlebox, in single cropping and in intercropping.

Additionally, other scenarios can be simulated by the expression $Xo = \sqrt[b]{2\ (t_1 + t_2)^2 CV^2 / rd^2}$ (HATHEWAY, 1961), based on the mean of the soil heterogeneity index (b) of the function of Smith (1938) and on the mean of the coefficient of variation (CV) of

FM, of the three trials of each composition. Thus, the following estimates would be used for the compositions: 100% M (b=1.0330; CV=12.58%), 75% M + 25% SLR (b=1.4709; CV=12.24%), 50% M + 50% SLR (b=0.9183; CV=15.05%), 25% M + 75% SLR (b=0.9535; CV=15.72%) and 100% SLR (b=1.1444; CV=14.74%) (Tables 2 and 3).

In this context, as an example, to evaluate the FM of eight treatments of the composition 50% M + 50% SLR, with five replicates and with d=10%, in the RCBD, there is: b=0.9183; DF=(8-1)(5-1)=28; t_1=INVT(0.05;28)=2.048407115; t_2=INVT(0.40;28)=0.85464749; CV=15.05%; r=5; d=10%. Therefore, $Xo = \sqrt[0.9183]{2(2.048407115+0.85464749)^2 15.05^2/5\times10^2}$ = 9.15 BEU. In the CRD, there is: b=0.9183; DF=(8)(5-1)=32; t_1=INVT(0.05;32)=2.036933334; t_2=INVT(0.40;32)=0.85299845; CV=15.05%; r=5; d=10%. Therefore,

$$Xo = \sqrt[0.9183]{2(2.036933334+0.85299845)^2 15.05^2/5\times10^2}$$

= 9.06 BEU. Thus, using the criterion of approximation to the next integer, the plot size for this example would be 10 m^2.

The results of this study serve as a reference for the definition of plot size and the number of replicates in experiments to evaluate the fresh matter of millet and slender leaf rattlebox, in single cropping or intercropping, in experiments conducted in CRD and RCBD. The use of plots of 10 m^2 is recommended due to the practical feasibility in the field and the stabilization of precision from this size. Additionally, it is an intermediate size, that is, slightly larger than the sizes determined for millet (*Pennisetum glaucum* L.), cv. 'Comum' (BURIN *et al*., 2015, 2016) and for sunn hemp (*Crotalatia juncea*) (FACCO *et al*., 2017, 2018) and smaller than those used by Ascari *et al*. (2020), Debiasi *et al*. (2016), Ferreira *et al*. (2019), Nascente; Stone (2018), Passos *et al*. (2017), Pfüller *et al*. (2019), Sousa *et al*. (2017) and Vuicik *et al*. (2018), in studies with millet and slender leaf rattlebox, along with other soil cover species.

CONCLUSIONS

In experiments to evaluate the fresh matter of millet and slender leaf rattlebox, in single cropping or intercropping, in completely randomized or randomized complete block designs, with 5 to 20 treatments and with five replications, plots of 10 m² of usable area are sufficient for differences between treatments of 10% of the overall mean of the experiment to be considered significant at 0.05 probability level.

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