







Experimental dimensions and precision in trials with millet and showy rattlebox

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ABSTRACT: The objective of this study was to determine the optimal plot size to evaluate the mass of fresh matter in millet (*Pennisetum glaucum* L.) and showy rattlebox (*Crotalaria spectabilis* Roth.), in scenarios formed by combinations of treatment numbers, repetitions numbers, and levels of experimental precision. Fifteen uniformity trials with millet and showy rattlebox, in single or intercropping, were carried out. The mass of fresh matter was evaluated in 540 basic experimental units (BEU) of 1 × 1 m (15 trials × 36 BEU per trial). The heterogeneity index of Smith (1938) was estimated. The plot size was determined by the method of Hatheway (1961) in scenarios formed by combinations of *i* treatments (*i* = 5, 10, 15, 20, and 25), *r* repetitions (*r* = 3, 4, 5, 6, 7, 8, 9, and 10), and *d* precision levels (*d* = 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20%). To evaluate the mass of fresh matter of millet and showy rattlebox, in single or intercropping, with 5 to 25 treatments and with five repetitions, plots of 10 m² of useful area are sufficient for differences between treatments of 9% of the overall average of the experiment to be considered significant at 0.05 probability.

Key words: *Crotalaria spectabilis* Roth.; optimal plot size; *Pennisetum glaucum* L.; repetitions numbers; soil cover crop

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

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 spectabilis (*Crotalaria spectabilis* Roth.) 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 spectabilis, em cultivo solteiro e em consórcio. Foi avaliada a massa de matéria fresca em 540 unidades experimentais básicas (UEB) de 1 × 1 m (15 ensaios × 36 UEB por ensaio). Foi estimado o índice de heterogeneidade 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, 20 e 25), *r* repetições (*r* = 3, 4, 5, 6, 7, 8, 9 e 10) e *d* níveis de precisão (*d* = 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 e 20%). Para avaliar a massa de matéria fresca de milheto e de crotalária spectabilis, em cultivo solteiro ou em consórcio, com 5 a 25 tratamentos e com cinco repetições, parcelas de 10 m² de área útil são suficientes para que diferenças entre tratamentos de 9% da média geral do experimento sejam consideradas significativas a 0,05 de probabilidade.

Palavras-chave: *Crotalaria spectabilis*; tamanho ótimo de parcela; *Pennisetum glaucum* L.; número de repetições; cultura de cobertura de solo

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Introduction

Millet (*Pennisetum glaucum* L.) and showy rattlebox (*Crotalaria spectabilis* Roth.) have been studied with respect to soil cover rate, decomposition rate, nutrient content, and phytomass production (Passos et al., 2017; Scavazza et al., 2018; Ferreira et al., 2019; Pfüller et al., 2019). Also, the effects on soil chemical and physical properties (Passos et al., 2017; Sousa et al., 2017), nematodes (Debiasi et al., 2016; Nascimento et al., 2020), weeds (São Miguel et al., 2018) and, consequently, on soybean (Debiasi et al., 2016; São Miguel et al., 2018) and okra (Nascimento et al., 2020) productivity have been investigated. In these researches, beneficial aspects of these ground cover species in single and intercropping were pointed out.

Such experiments were conducted with three repetitions and plots of 24 m² (Ferreira et al., 2019), four repetitions and plots of 10 m² (Nascimento et al., 2020); 12 m² (Pfüller et al., 2019); 50 m² (Passos et al., 2017); 60 m² (Debiasi et al., 2016); 63 m² (São Miguel et al., 2018); and 150 m² (Sousa et al., 2017) and six repetitions and plots of 30 m² (Scavazza et al., 2018). However, the criteria used to define the plot size and the number of repetitions were not mentioned.

From the uniformity trial data (trials without treatments) it is possible to apply Smith (1938) and Hatheway (1961) methodologies to calculate the optimal plot size according to the experimental design, the number of treatments, the number of repetitions, and the experimental precision. These methodologies have been used in sunflower (Sousa et al., 2016), in banana (Donato et al., 2018), in forage palm (Guimarães et al., 2020) and in species with potential for ground cover, such as: velvet bean (Cargnelutti Filho et al., 2014a); forage turnip (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 single cropping of millet (*Pennisetum glaucum* L.), cv. comum (Burin et al., 2015, 2016) and sunn hemp (*Crotalaria juncea* L.) (Facco et al., 2017) by averages of the maximum curvature method of the coefficient of variation model (Paranaíba et al., 2009). It is assumed that intercropping, commonly used with ground covers, can generate different experimental design patterns and that the use of Smith (1938) and Hatheway (1961) methodologies with another millet cultivar and another crotalaria species can add important information to the planning of experiments with these two ground covers.

Thus, the objective of this work was to determine the optimal plot size for evaluating the mass of fresh matter of millet (*Pennisetum glaucum* L.) and showy rattlebox (*Crotalaria spectabilis*), in scenarios formed by combinations of treatment numbers, repetitions numbers, and levels of experimental precision.

Materials and Methods

Fifteen uniformity trials with millet (*Pennisetum glaucum* L.), cultivar BRS 1501 (M), and showy rattlebox (*Crotalaria*

spectabilis) (CS), were conducted in an experimental area located at 29° 42' S, 53° 49' W and 95 m altitude. At this site, the climate is Cfa humid subtropical, according to Köppen classification, with hot summers and no dry season (Alvares et al., 2013) and the soil is Arenic Dystrophic Red Argissolo (Santos et al., 2018). Physical and chemical analysis of the soil at a depth of 0 - 20 cm 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; CTC_{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 (repetitions) were conducted of each of the following five compositions, with the respective seeding densities in parentheses: 100% M (25 kg ha⁻¹); 75% M (18.75 kg ha⁻¹) + 25% CS (4.6875 kg ha⁻¹); 50% M (12.5 kg ha⁻¹) + 50% CS (9.375 kg ha⁻¹); 25% M (6.25 kg ha⁻¹) + 75% CS (14.0625 kg ha⁻¹); and 100% CS (18.75 kg ha⁻¹). On November 13, 2019, base fertilization was performed, with 20 kg ha⁻¹ of N, 80 kg ha⁻¹ of P₂O₅ and 80 kg ha⁻¹ of K₂O (N-P-K, formulation 05-20-20) and 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 of size 6 × 6 m (36 m²) was divided into 36 basic experimental units (BEU) of 1 × 1 m (1 m²), forming a matrix of six rows and six columns. On February 3rd and 4th, 2020, at the flowering of millet plants, in each BEU, the plants were cut, close to the soil surface, and the mass of fresh matter (FM) was weighed, in g m⁻². It was decided to cut the millet at flowering, to minimize the effects on the mass of fresh matter due to leaf senescence of the crop after this period. 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 distinct sizes and/or shapes were planned as (X=X_R×X_C), i.e., (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 abbreviations X_R, X_C and X, stand for number of adjacent BEU in the row, number of adjacent BEU in the column and plot size in number of BEU, respectively.

For each plot size (X) were determined: n - number of plots with X BEU of size (n=36/X); M_(X) - average of the plots with X BEU size; V_(X) - variance among plots of X BEU size; CV_(X) - coefficient of variation (in %) among plots of X BEU size; and VU_(X) - variance per BEU among the plots of X BEU size [VU_(X)=V_(X)/X²].

The parameters V1 (variance per BEU among the plots of a BEU size) and b (index of heterogeneity) and the coefficient of determination (r²) 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, i.e., logVU_(X) = logV1 - b logX, whose estimation was weighted by the degrees of freedom (DF=n-1), associated with each plot size, as applied by Sousa et al. (2016). The

observed values of the dependent [$VU_{(x)}$] and independent (X) variables and the function $VU_{(x)}=V1/X^b$ (Smith, 1938) were plotted graphically.

Experimental plans were simulated for the scenarios formed by combinations of i treatments ($i = 5, 10, 15, 20$, and 25), r repetitions ($r = 3, 4, 5, 6, 7, 8, 9$, and 10) and d differences between treatment averages to be detected as significant at 0.05 probability, expressed as a percentage of the overall average of the experiment, i.e., in levels of precision [$d = 4\%$ (highest precision), 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20% (lowest precision)]. For each experimental plan, the optimal plot size (X_0), in number of BEU, was calculated using the expression

$$X_0 = b \sqrt{\frac{2(t_1 + t_2)^2 CV^2}{rd^2}} \quad (\text{Hatheway, 1961}).$$

In this expression b is the estimate of the heterogeneity index (in this study, the average of b from the 15 uniformity trials was taken); t_1 is the critical value of Student's t distribution for the significance level of the test (type I error) of $\alpha = 5\%$ (two-sided test at 5%), with DF degrees of freedom; t_2 is the critical value of Student's t distribution, corresponding to $2(1-P)$ (two-sided test), where P is the probability of obtaining a significant result, 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 among plots of a BEU size (in this study, the average CV of the 15 uniformity trials was taken), in percent; r is the number of repetitions and d is the difference between treatment averages to be detected as significant at 0.05 probability, expressed as a percentage of the overall average of the experiment (precision). The degrees of freedom (DF) for obtaining the critical values (tabulated) of Student's t -distribution were obtained by the expression $DF=(i)(r-1)$, where i is the number of treatments and r is the number of repetitions. The values of t_1 and t_2 , in this study, were obtained with the Microsoft Office Excel® software, through the functions $t_1=INVT(0.05;DF)$ and $t_2=INVT(0.40;DF)$, respectively.

The data of FM, CV and b , obtained in the three uniformity trials (repetitions) of each of the five compositions, were submitted to analysis of variance and Scott Knott test via bootstrap with 10,000 resamples, with the aid of Sisvar software (Ferreira, 2014). These statistical procedures are suitable to circumvent possible impacts of not meeting the assumptions of normality of errors and homogeneity of residual variances (Ferreira, 2014). The other statistical analyses were performed with the help of Microsoft Office Excel® software.

Results and Discussion

In the 15 uniformity trials, formed by compositions of sowing densities of millet (*Pennisetum glaucum* L.), cultivar BRS 1501 (M) and showy rattlebox (*Crotalaria spectabilis*

Roth.) (CS), the mass of fresh matter (FM) ranged between 4382 and 8276 $g\ m^{-2}$, i.e., 43.82 and 82.76 $Mg\ ha^{-1}$, respectively (Table 1). The average FM, from the three trials for each composition were 7117, 7442, 7861, 7955, and 4593 $g\ m^{-2}$, for the compositions of 100% M, 75% M + 25% CS, 50% M + 50% CS, 25% M + 75% CS, and 100% CS, respectively. Two groups of averages were formed by Scott Knott bootstrap test at 5% significance level. The average FM of the composition with millet alone and of the compositions in intercropping, did not differ, and were higher than the FM produced by showy rattlebox in single cropping. For these same millet and showy rattlebox cultivars, 34.59 and 33.9 $Mg\ ha^{-1}$ of FM were obtained by Passos et al. (2017) and 5.327 and 1.67 $Mg\ ha^{-1}$ by Pfüller et al. (2019), respectively.

The coefficient of variation (CV) of FM, obtained among the 36 BEU in each of the 15 uniformity trials, ranged from 9.87 to 18.51%, with an average of 14.62% (Table 1). The average CV, of the three trials of each composition, was 14.49, 14.82, 15.85, 14.85, and 13.07%, for the compositions of 100% M, 75% M + 25% CS, 50% M + 50% CS, 25% M + 75% CS, and 100% CS, respectively, and by the F-test of the analysis of variance, they did not differ (p -value = 0.7599). This suggests that experiments with millet and showy rattlebox, in

Table 1. Mass of fresh matter (FM), coefficient of variation (CV) and index of heterogeneity (b) of Smith (1938), in three uniformity trials (repetitions) of each of the five compositions of millet (M) and showy rattlebox (CS) sowing densities. F-test value and respective p -value of the analysis of variance via bootstrap with 10,000 resamples, for FM, CV and b .

Composition	Trial ⁽¹⁾	FM ($g\ m^{-2}$) ⁽²⁾	CV (%) ⁽³⁾	b ⁽³⁾
100% M	1	6979	16.03	0.6828
	2	6841	13.76	0.5453
	3	7532	13.68	0.6995
	Average	7117 a	14.49	0.6425
75% M + 25% CS	1	6993	15.89	1.0487
	2	7181	16.30	0.7891
	3	8151	12.26	1.0379
	Average	7442 a	14.82	0.9586
50% M + 50% CS	1	8276	18.51	1.3545
	2	7465	13.90	0.9625
	3	7843	15.14	0.6280
	Average	7861 a	15.85	0.9817
25% M + 75% CS	1	7827	13.27	1.1609
	2	8234	13.53	1.3363
	3	7804	17.76	1.2871
	Average	7955 a	14.85	1.2615
100% CS	1	4382	17.07	0.7250
	2	4656	12.28	1.0911
	3	4742	9.87	1.0725
	Average	4593 b	13.07	0.9629
	Overall average	6994	14.62	0.9614
F		36.889	0.468	3.404
p -value		0.0013	0.7599	0.0635

⁽¹⁾ Each uniformity trial of size $6 \times 6\ m$ ($36\ m^2$) was divided into 36 BEU of $1 \times 1\ m$ ($1\ m^2$), forming a matrix of six rows and six columns. ⁽²⁾ Averages not followed by the same letter in the column (comparison of averages between compositions) differ at 5% significance by Scott Knott bootstrap test with 10,000 re-samples. ⁽³⁾ The averages of the compositions do not differ ($p > 0.05$).

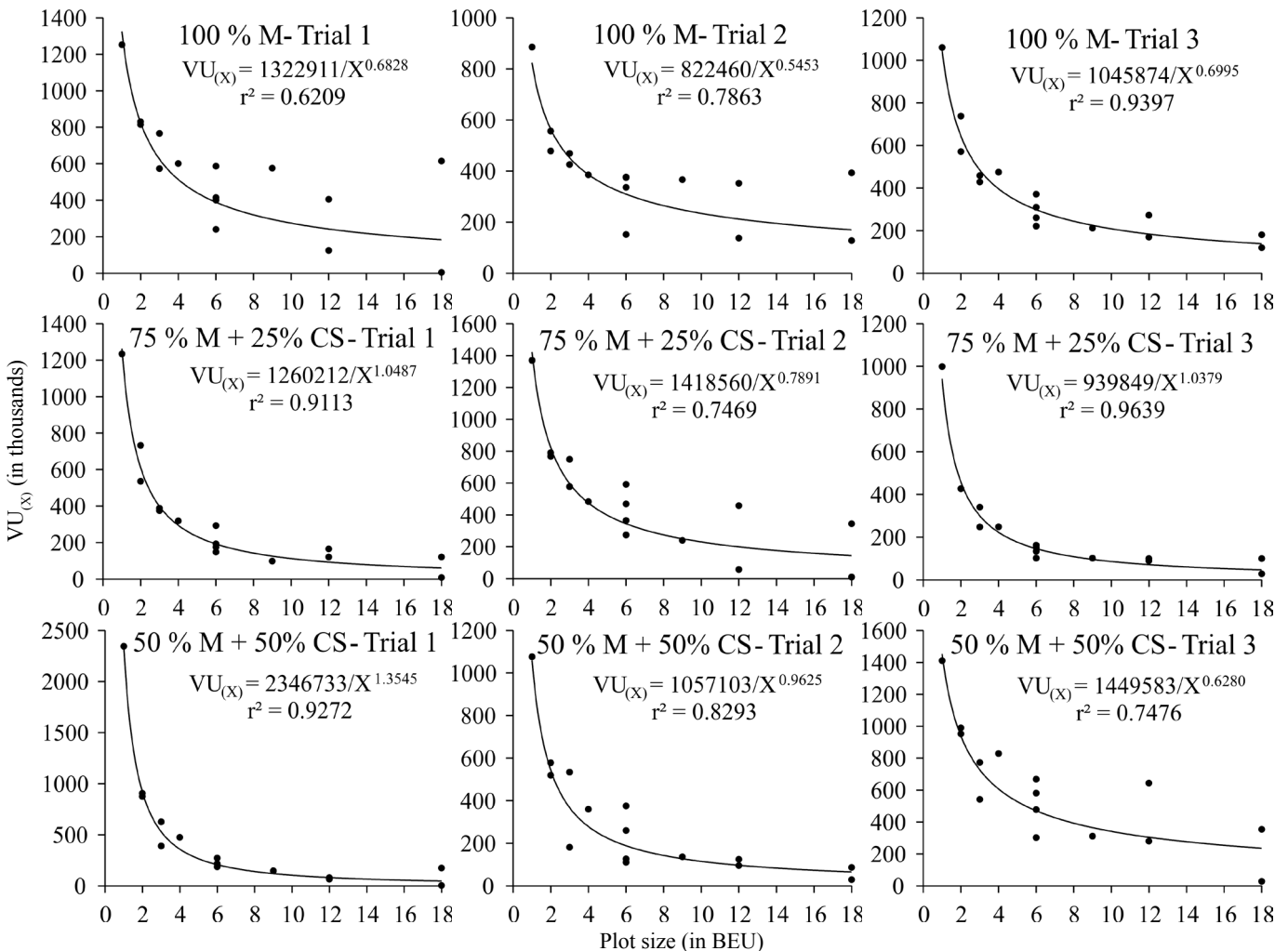
single cropping or in intercropping, have similar experimental accuracy. Additionally, it can be inferred that using the average CV of the 15 trials (CV = 14.62%), in Hatheway (1961) methodology, is adequate to represent all compositions.

Smith (1938) heterogeneity index (b) among the 15 uniformity trials ranged from 0.5453 to 1.3545, with an average of 0.9614 (Table 1). The averages of b, from the three trials for each composition were 0.6425, 0.9586, 0.9817, 1.2615, and 0.9629, for the compositions of 100% M, 75% M + 25% CS, 50% M + 50% CS, 25% M + 75% CS, and 100% CS, respectively, and did not differ (p-value = 0.0635). Then, one can use the average b of the 15 trials (b = 0.9614) in Hatheway (1961) methodology, to represent the five compositions. Values of b close to unity indicate high heterogeneity or low correlation between adjacent plots. According to Lin & Binns (1986), when $b > 0.7$, it is recommended to increase the plot size, when $b < 0.2$, one should increase the number of repetitions, and in cases of $0.2 \leq b \leq 0.7$ it is appropriate to investigate the best combination of plot size and number of repetitions. Thus, it can be inferred that in experiments of millet and showy rattlebox, in single cultivation or in intercropping, one should prioritize the use of larger size plots.

In the 15 uniformity trials, there was a decrease in the variance per BEU among plots [$VU_{(x)}$], which indicates

improvement in experimental precision with increasing planned plot size (X) (Figure 1). The decreases were steep up to plots four BEU in size (4 m²), intermediate between four and ten BEU, and a stabilizing trend with plots larger than ten BEU. Similar pattern to this was observed in velvet bean (Cargnelutti Filho et al., 2014a); forage turnip (Cargnelutti Filho et al., 2014b); flax (Cargnelutti Filho et al., 2018); and black oats with common vetch (Cargnelutti Filho et al., 2020). So, to evaluate the mass of fresh matter of millet and showy rattlebox, in single cultivation or in intercropping, a plot size of up to 10 m² is indicated. This value is relatively higher than the optimal plot size required to evaluate the mass of fresh matter of millet, cv. common, which was 4.46 m² in three evaluation seasons (Burin et al., 2015) and 4.97 m², for the three sowing and cutting seasons (Burin et al., 2016). It was also larger than the 2.04 m² size (Facco et al., 2017) to evaluate the mass of fresh matter of sunn hemp. Differences among environments, millet cultivars, crotalaria species, and methodologies used for plot size determination contribute to explain the different results from those obtained in this study.

In Hatheway methodology (1961), from fixed values of the coefficient of variation (CV = 14.62%) and Smith heterogeneity index (1938) (b = 0.9614), it is possible to determine different optimal plot sizes (X_0), as a function of the number of



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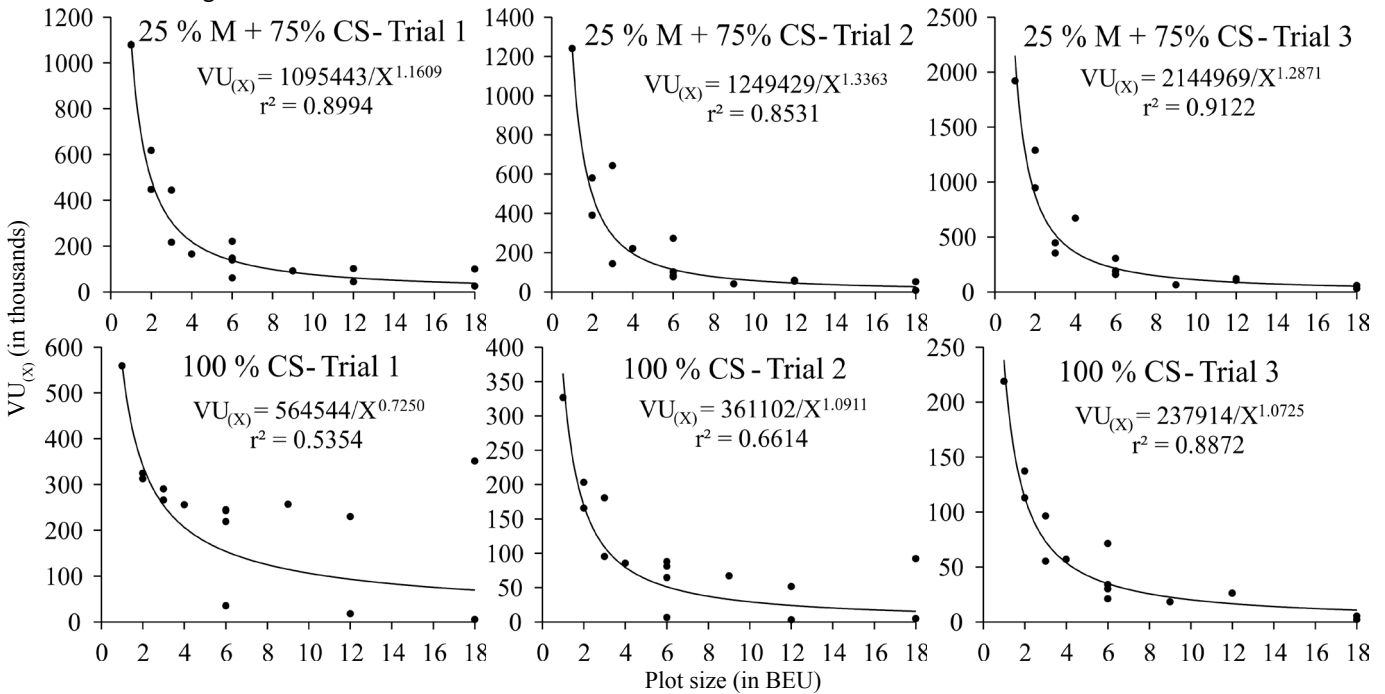


Figure 1. Relationship between the variance per basic experimental unit (BEU) between X BEU plot sizes [$VU_{(x)} = V_{(x)}/X^2$], in thousands, and the planned plot size (X), in BEU, and the parameter estimates of the function $VU_{(x)} = V_1/X^b$ of Smith (1938). Mass of fresh matter data obtained 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 showy rattlebox (*Crotalaria spectabilis*), common cultivar (CS).

treatments (i), the number of repetitions (r), and precision m², the researcher can investigate within his availability of (d) (Table 2). Therefore, besides the indicated size of 10 experimental area, number of treatments to be evaluated and

Table 2. Optimum plot size, in m², in combinations of i treatments, r repetitions and d precision levels (%), for mass of fresh matter in compositions of millet and showy rattlebox sowing densities (CV = 14.62%; heterogeneity index b = 0.9614).

i	r	d (%)																	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
5	3	102.8	64.6	44.2	32.1	24.3	19.0	15.3	12.5	10.5	8.9	7.6	6.6	5.7	5.1	4.5	4.0	3.6	
	4	70.7	44.5	30.4	22.1	16.7	13.1	10.5	8.6	7.2	6.1	5.2	4.5	4.0	3.5	3.1	2.8	2.5	
	5	54.1	34.0	23.3	16.9	12.8	10.0	8.0	6.6	5.5	4.7	4.0	3.5	3.0	2.7	2.4	2.1	1.9	
	6	43.8	27.5	18.8	13.7	10.4	8.1	6.5	5.3	4.5	3.8	3.2	2.8	2.4	2.2	1.9	1.7	1.5	
	7	36.8	23.1	15.8	11.5	8.7	6.8	5.5	4.5	3.7	3.2	2.7	2.4	2.1	1.8	1.6	1.4	1.3	
	8	31.7	19.9	13.6	9.9	7.5	5.9	4.7	3.9	3.2	2.7	2.3	2.0	1.8	1.6	1.4	1.2	1.1	
	9	27.8	17.5	12.0	8.7	6.6	5.2	4.1	3.4	2.8	2.4	2.1	1.8	1.6	1.4	1.2	1.1	1.0	
	10	24.8	15.6	10.7	7.7	5.9	4.6	3.7	3.0	2.5	2.1	1.8	1.6	1.4	1.2	1.1	1.0	0.9	
	10	3	92.0	57.9	39.6	28.7	21.8	17.0	13.7	11.2	9.4	7.9	6.8	5.9	5.1	4.5	4.0	3.6	3.2
		4	65.8	41.4	28.3	20.6	15.6	12.2	9.8	8.0	6.7	5.7	4.9	4.2	3.7	3.2	2.9	2.6	2.3
5		51.3	32.3	22.1	16.0	12.1	9.5	7.6	6.3	5.2	4.4	3.8	3.3	2.9	2.5	2.2	2.0	1.8	
6		42.0	26.4	18.1	13.1	9.9	7.8	6.2	5.1	4.3	3.6	3.1	2.7	2.3	2.1	1.8	1.6	1.5	
7		35.5	22.3	15.3	11.1	8.4	6.6	5.3	4.3	3.6	3.1	2.6	2.3	2.0	1.8	1.6	1.4	1.2	
8		30.8	19.3	13.2	9.6	7.3	5.7	4.6	3.8	3.1	2.7	2.3	2.0	1.7	1.5	1.3	1.2	1.1	
9		27.1	17.1	11.7	8.5	6.4	5.0	4.0	3.3	2.8	2.3	2.0	1.7	1.5	1.3	1.2	1.1	1.0	
10		24.2	15.2	10.4	7.6	5.7	4.5	3.6	3.0	2.5	2.1	1.8	1.6	1.4	1.2	1.1	0.9	0.9	
15		3	88.8	55.8	38.2	27.7	21.0	16.4	13.2	10.8	9.0	7.6	6.6	5.7	5.0	4.4	3.9	3.5	3.1
		4	64.3	40.4	27.7	20.1	15.2	11.9	9.6	7.8	6.5	5.5	4.7	4.1	3.6	3.2	2.8	2.5	2.3
	5	50.4	31.7	21.7	15.7	11.9	9.3	7.5	6.1	5.1	4.3	3.7	3.2	2.8	2.5	2.2	2.0	1.8	
	6	41.4	26.0	17.8	12.9	9.8	7.7	6.2	5.1	4.2	3.6	3.1	2.6	2.3	2.0	1.8	1.6	1.5	
	7	35.1	22.1	15.1	11.0	8.3	6.5	5.2	4.3	3.6	3.0	2.6	2.2	2.0	1.7	1.5	1.4	1.2	
	8	30.5	19.2	13.1	9.5	7.2	5.6	4.5	3.7	3.1	2.6	2.2	1.9	1.7	1.5	1.3	1.2	1.1	
	9	26.9	16.9	11.6	8.4	6.4	5.0	4.0	3.3	2.7	2.3	2.0	1.7	1.5	1.3	1.2	1.1	0.9	
	10	24.1	15.1	10.3	7.5	5.7	4.5	3.6	2.9	2.4	2.1	1.8	1.5	1.3	1.2	1.1	0.9	0.8	

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i	r	d (%)																	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
20	3	87.3	54.9	37.5	27.2	20.6	16.2	13.0	10.6	8.9	7.5	6.4	5.6	4.9	4.3	3.8	3.4	3.1	
	4	63.6	40.0	27.4	19.9	15.0	11.8	9.5	7.8	6.5	5.5	4.7	4.1	3.6	3.1	2.8	2.5	2.2	
	5	50.0	31.4	21.5	15.6	11.8	9.3	7.4	6.1	5.1	4.3	3.7	3.2	2.8	2.5	2.2	2.0	1.8	
	6	41.1	25.9	17.7	12.8	9.7	7.6	6.1	5.0	4.2	3.5	3.0	2.6	2.3	2.0	1.8	1.6	1.4	
	7	34.9	22.0	15.0	10.9	8.3	6.5	5.2	4.3	3.6	3.0	2.6	2.2	2.0	1.7	1.5	1.4	1.2	
	8	30.3	19.1	13.0	9.5	7.2	5.6	4.5	3.7	3.1	2.6	2.2	1.9	1.7	1.5	1.3	1.2	1.1	
	9	26.8	16.8	11.5	8.4	6.3	5.0	4.0	3.3	2.7	2.3	2.0	1.7	1.5	1.3	1.2	1.0	0.9	
	10	24.0	15.1	10.3	7.5	5.7	4.4	3.6	2.9	2.4	2.1	1.8	1.5	1.3	1.2	1.0	0.9	0.8	
	25	3	86.4	54.3	37.2	27.0	20.4	16.0	12.8	10.5	8.8	7.4	6.4	5.5	4.8	4.3	3.8	3.4	3.0
		4	63.2	39.7	27.2	19.7	14.9	11.7	9.4	7.7	6.4	5.4	4.7	4.0	3.5	3.1	2.8	2.5	2.2
5		49.7	31.3	21.4	15.5	11.8	9.2	7.4	6.1	5.1	4.3	3.7	3.2	2.8	2.5	2.2	1.9	1.7	
6		41.0	25.8	17.6	12.8	9.7	7.6	6.1	5.0	4.2	3.5	3.0	2.6	2.3	2.0	1.8	1.6	1.4	
7		34.8	21.9	15.0	10.9	8.2	6.4	5.2	4.2	3.5	3.0	2.6	2.2	1.9	1.7	1.5	1.4	1.2	
8		30.2	19.0	13.0	9.4	7.1	5.6	4.5	3.7	3.1	2.6	2.2	1.9	1.7	1.5	1.3	1.2	1.1	
9		26.7	16.8	11.5	8.3	6.3	4.9	4.0	3.3	2.7	2.3	2.0	1.7	1.5	1.3	1.2	1.0	0.9	
10		23.9	15.0	10.3	7.5	5.7	4.4	3.6	2.9	2.4	2.1	1.8	1.5	1.3	1.2	1.0	0.9	0.8	

desired precision, which combination of plot size and number of repetitions is the most appropriate.

With fixed values of i and r , X_0 increased with increasing accuracy (d) (Table 2). For example, to evaluate FM in an experiment conducted in completely randomized design (CRD), with five treatments and three repetitions, aiming that in 80% of the experiments (power = 0.80) differences between treatments of $d = 20\%$ of the overall average of the experiment (lower precision) are detected as significant at 5% probability, the plot size should be 3.6 BEU (3.6 m²) (Table 2). At the other extreme, i.e. plots of 102.8 m² would make it possible to improve accuracy and obtain $d = 4\%$. However, conducting a field experiment with a 102.8 m² plot requires a larger experimental area and can make the experiment difficult to execute. In practice, high experimental accuracies (low percentages of d) are difficult to achieve because of the need for high plot size, as already pointed out by Cargnelutti Filho et al. (2014a, 2014b, 2018, 2020). Additionally, with fixed values of i and d , X_0 decreased with the increase of r , and with fixed values of r and d , X_0 decreased with the increase of i . Similar pattern has been found by Cargnelutti Filho et al. (2014a, 2014b, 2018, 2020).

The information from this study enables investigations into 480 scenarios formed by combinations of i treatments ($i = 5, 10, 15, 20$, and 25), r repetitions ($r = 3, 4, 5, 6, 7, 8, 9$, and 10) and d differences between treatment averages to be detected as significant at 5% probability ($d = 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19$, and 20%). For example, if the researcher wants to evaluate the FM of five treatments, in CRD, and wants precision (d) of 10%, among the various options, he could use plots of 15.3 BEU (15.3 m²) and three repetitions, 10.5 BEU (10.5 m²) and four repetitions, 8.0 BEU (8.0 m²) and five repetitions, 6.5 BEU (6.5 m²) and six repetitions, 5.5 BEU (5.5 m²) and seven repetitions, 4.7 BEU (4.7 m²) and eight repetitions, 4.1 BEU (4.1 m²) and nine repetitions and 3.7 BEU (3.7 m²) and ten repetitions (Table 2). In this situation, the required experimental area is 229, 210, 201, 195, 191, 188, 186, and 184 m², respectively (Table 3).

Other scenarios can be simulated using the expression

$$X_0 = b \sqrt{\frac{2(t_1 + t_2)^2 CV^2}{rd^2}} \quad (\text{Hatheway, 1961}).$$

For example, to evaluate the FM of eight treatments, with five repetitions and with $d=9\%$, in CRD, one has: $b=0.9614$; $DF=(8)(5-1)=32$; $t_1=INVT(0.05;32)=2.036933334$; $t_2=INVT(0.40;32)=0.85299845$; $CV=14.62\%$; $r=5$; $d=9\%$. Therefore,

$$X_0 = 0.9614 \sqrt{\frac{2(2.036933334 + 0.85299845)^2 14.62^2}{5 \cdot 9^2}} = 9.6 \text{ BEU.}$$

If the researcher wants to conduct the experiment in a randomized complete block design, he has: $b=0.9614$; $DF=(8-1)(5-1)=28$; $t_1=INVT(0.05;28)=2.048407115$; $t_2=INVT(0.40;28)=0.85464749$; $CV=14.62\%$; $r=5$; $d=9\%$. Therefore,

$$X_0 = 0.9614 \sqrt{\frac{2(2.048407115 + 0.85464749)^2 14.62^2}{5 \cdot 9^2}} = 9.7 \text{ BEU.}$$

Therefore, using the criterion of rounding up to the nearest whole number to ensure the desired precision, for these examples, the plot size would be 10 m² and the experimental area 400 m².

The results of this study serve as a reference for defining the plot size and the number of repetitions in experiments to evaluate the mass of fresh matter of millet and showy rattlebox, cultivated alone or in intercropping. In other cultures, such as: sunflower (Sousa et al., 2016); banana (Donato et al., 2018); forage palm (Guimarães et al., 2020); velvet bean (Cargnelutti Filho et al., 2014a); forage turnip (Cargnelutti Filho et al., 2014b); flax (Cargnelutti Filho et al.,

Table 3. Experiment size, in m², in combinations of i treatments, r repetitions and d precision levels (%), for mass of fresh matter in compositions of millet and showy rattlebox sowing densities (CV = 14.62%; heterogeneity index b = 0.9614).

i	r	d (%)																	
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
5	3	1542	970	664	481	365	285	229	188	157	133	114	99	86	76	67	60	54	
	4	1415	890	609	442	335	262	210	173	144	122	104	90	79	70	62	55	50	
	5	1352	850	582	422	320	250	201	165	138	116	100	86	76	67	59	53	48	
	6	1314	826	565	410	311	243	195	160	134	113	97	84	73	65	58	51	46	
	7	1288	810	554	402	305	238	191	157	131	111	95	82	72	63	56	50	45	
	8	1268	797	546	396	300	235	188	155	129	109	94	81	71	63	55	50	45	
	9	1253	787	539	391	296	232	186	153	127	108	92	80	70	62	55	49	44	
	10	1240	780	534	387	293	230	184	151	126	107	92	79	69	61	54	49	44	
	10	3	2761	1736	1188	862	653	511	410	337	281	238	204	177	154	136	121	108	97
		4	2634	1656	1133	822	623	487	392	321	268	227	194	168	147	130	115	103	93
5		2565	1613	1104	801	607	475	381	313	261	221	189	164	143	126	112	100	90	
6		2520	1584	1084	787	596	466	375	307	256	217	186	161	141	124	110	99	89	
7		2487	1564	1070	776	588	460	370	303	253	214	184	159	139	123	109	97	87	
8		2462	1548	1059	769	582	456	366	300	250	212	182	157	138	121	108	96	87	
9		2441	1535	1050	762	577	452	363	298	248	210	180	156	137	120	107	95	86	
10		2424	1524	1043	757	573	449	360	296	247	209	179	155	136	119	106	95	85	
15		3	3997	2513	1719	1248	945	740	594	487	407	344	295	256	223	197	175	156	140
		4	3860	2427	1661	1205	913	714	574	471	393	332	285	247	216	190	169	151	136
	5	3782	2377	1627	1181	894	700	562	461	385	326	279	242	211	186	166	148	133	
	6	3728	2344	1604	1164	882	690	554	455	379	321	275	238	208	184	163	146	131	
	7	3688	2319	1587	1151	872	683	548	450	375	318	272	236	206	182	161	144	130	
	8	3657	2299	1573	1142	865	677	544	446	372	315	270	234	204	180	160	143	129	
	9	3631	2282	1562	1133	859	672	540	443	369	313	268	232	203	179	159	142	128	
	10	3608	2268	1552	1126	853	668	536	440	367	311	266	231	202	178	158	141	127	
	20	3	5237	3292	2253	1635	1238	969	778	638	533	451	387	335	293	258	229	205	184
		4	5088	3198	2189	1588	1203	942	756	620	518	438	376	325	284	251	223	199	179
5		4999	3143	2151	1561	1182	925	743	609	509	431	369	320	280	246	219	196	176	
6		4937	3104	2124	1541	1167	914	734	602	502	425	364	316	276	243	216	193	174	
7		4890	3074	2104	1527	1156	905	727	596	497	421	361	313	273	241	214	191	172	
8		4852	3050	2087	1515	1147	898	721	592	494	418	358	310	271	239	212	190	171	
9		4820	3030	2074	1505	1140	892	717	588	490	415	356	308	270	238	211	189	169	
10		4793	3013	2062	1496	1133	887	712	584	488	413	354	307	268	236	210	187	168	
25		3	6478	4072	2787	2022	1532	1199	963	790	659	558	478	414	362	319	284	253	228
		4	6316	3970	2717	1972	1493	1169	939	770	643	544	466	404	353	311	276	247	222
	5	6217	3908	2674	1941	1470	1151	924	758	632	535	459	398	348	306	272	243	219	
	6	6146	3864	2644	1919	1453	1138	914	749	625	529	454	393	344	303	269	240	216	
	7	6092	3829	2621	1902	1440	1127	906	743	620	525	450	390	341	300	267	238	214	
	8	6047	3802	2602	1888	1430	1119	899	737	615	521	446	387	338	298	265	237	213	
	9	6010	3778	2586	1876	1421	1112	893	733	611	518	444	384	336	296	263	235	211	
	10	5978	3758	2572	1866	1414	1106	889	729	608	515	441	382	334	295	262	234	210	

2018); and black oats with common vetch (Cargnelutti Filho et al., 2020), the application of Smith (1938) and Hatheway (1961) methodologies has also generated subsidies for planning experiments.

It is indicated to use plots of 10 m², due to practical feasibility in the field and the stabilization of accuracy from this size that is higher than those established for millet (*Pennisetum glaucum* L.), cv. comum (Burin et al., 2015, 2016) and for *C. juncea* (Facco et al., 2017) and lower than or equal to those used in the experiments with millet and showy rattlebox, along with other ground cover species, by Debiasi et al. (2016), Passos et al. (2017), Sousa et al. (2017), São Miguel et al. (2018), Scavazza et al. (2018), Ferreira et al. (2019), Pfüller et al. (2019), and Nascimento et al. (2020).

Conclusions

In experiments to evaluate the mass of fresh matter of millet and showy rattlebox, in single cultivation or in intercrop, with 5 to 25 treatments and with five repetitions, plots of 10 m² of useful area are sufficient for differences between treatments of 9% of the overall average of the experiment to be considered significant at 0.05 probability.

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Literature Cited

- Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M.; Sparovek, G. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, v.22, n.6, p.711-728, 2013. <https://doi.org/10.1127/0941-2948/2013/0507>.
- Burin, C.; Cargnelutti Filho, A.; Alves, B.M.; Toebe, M.; Kleinpaul, J.A. Plot size and number of replicates in times of sowing and cuts of millet. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.20, n.2, p.119-127, 2016. <https://doi.org/10.1590/1807-1929/agriambi.v20n2p119-127>.
- Burin, C.; Cargnelutti Filho, A.; Alves, B.M.; Toebe, M.; Kleinpaul, J.A. Neu, I.M.M. Tamanho de parcela e número de repetições na cultura do milheto em épocas de avaliação. *Bragantia*, v.74, n.3, p.261-269, 2015. <https://dx.doi.org/10.1590/1678-4499.0465>.
- Cargnelutti Filho, A.; Neu, I.M.M.; Santos, G.O.; Facco, G.; Wartha, C.A.; Kleinpaul, J.A. Plot size related to numbers of treatments, repetitions, and the experimental precision in flax. *Comunicata Scientiae*, v.9, n.4, p.629-636, 2018. <https://doi.org/10.14295/cs.v9i4.1809>.
- Cargnelutti Filho, A.; Souza, J.M.; Pezzini, R.V.; Neu, I.M.M.; Silveira, D.L.; Procedi, A. Optimal plot size for experiments with black oats and the common vetch. *Ciência Rural*, v.50, n.3, e20190123, 2020. <https://doi.org/10.1590/0103-8478cr20190123>.
- Cargnelutti Filho, A.; Toebe, M.; Alves, B.M.; Burin, C.; Neu, I.M.M.; Facco, G. Tamanho de parcela para avaliar a massa de plantas de mucuna cinza. *Comunicata Scientiae*, v.5, n.2, p.196-204, 2014a. <http://comunicatascientiae.com.br/comunicata/article/view/328/245>. 04 Jun. 2020.
- Cargnelutti Filho, A.; Toebe, M.; Burin, C.; Casarotto, G.; Alves, B.M. Planejamentos experimentais em nabo forrageiro semeado a lanço e em linha. *Bioscience Journal*, v.30, n.3, p.677-686, 2014b. <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/18048/13925>. 04 Jun. 2020.
- Debiasi, H.; Franchini, J.C.; Dias, W.P.; Ramos Junior, E.U.; Balbinot Junior, A.A. Práticas culturais na entressafra da soja para o controle de *Pratylenchus brachyurus*. *Pesquisa Agropecuária Brasileira*, v.51, n.10, p.1720-1728, 2016. <https://doi.org/10.1590/s0100-204x2016001000003>.
- Donato, S.L.R.; Silva, J.A.; Guimarães, B.V.C.; Silva, S.O. Experimental planning for the evaluation of phenotypic descriptors in banana. *Revista Brasileira de Fruticultura*, v.40, n.5, p.1-13, 2018. <https://doi.org/10.1590/0100-29452018962>.
- Facco, G.; Cargnelutti Filho, A.; Alves, B.M.; Lavezo, A.; Follmann, D.N.; Bem, C.M.; Schabarum, D.E.; Kleinpaul, J.A.; Chaves, G.G.; Silveira, D.L.; Simões, F.M.; Uliana, D.B.; Wartha, C.A. Basic experimental unit and plot sizes with the method of maximum curvature of the coefficient of variation in sunn hemp. *African Journal of Agricultural Research*, v.12, n.6, p.415-423, 2017. <https://doi.org/10.5897/AJAR2016.11814>.
- Ferreira, D.F. Sisvar: a guide for its bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia*, v.38, n.2, p.109-112, 2014. <https://doi.org/10.1590/S1413-70542014000200001>.
- Ferreira, N.M.; Santos, D.; Bertino, A.M.P.; Cavalcante, A.C.P.; Pereira, W.E.; Bertino, A.M.P.; Oliveira, A.P. Potential of species of green coverage in Entisol. *Journal of Agricultural Science*, v.11, n.11, p.263-273, 2019. <https://doi.org/10.5539/jas.v11n11p263>.
- Guimarães, B.V.C.; Donato, S.L.R.; Aspiazú, I.; Azevedo, A.M.; Carvalho, A.J. Optimal plot size for experimental trials with *Opuntia cactus* pear. *Acta Scientiarum. Technology*, v.42, p.e42579, 2020. <https://doi.org/10.4025/actascitechnol.v42i1.42579>.
- Hatheway, W.H. Convenient plot size. *Agronomy Journal*, v.53, n.4, p.279-280, 1961. <https://doi.org/10.2134/agronj1961.00021962005300040025x>.
- Lin, C.S.; Binns, M.R. Relative efficiency of two randomized block designs having different plot sizes and numbers of replications and of plots per block. *Agronomy Journal*, v.78, n.3, p.531-534, 1986. <https://doi.org/10.2134/agronj1986.00021962007800030029x>.
- Nascimento, D.D.; Vidal, R.L.; Pimenta, A.A.; Castro, M.G.C.; Soares, P.L.M. Crotalaria and millet as alternative controls of root-knot nematodes infecting okra. *Bioscience Journal*, v.36, n.3, p.713-719, 2020. <http://dx.doi.org/10.14393/BJ-v36n3a2020-42248>.
- Paranaíba, P.F.; Ferreira, D.F.; Morais, A.R. Tamanho ótimo de parcelas experimentais: proposição de métodos de estimação. *Revista Brasileira de Biometria*, v.27, n.2, p.255-268, 2009. http://jaguar.fcav.unesp.br/RME/fasciculos/v27/v27_n2/Patricia.pdf. 04 Jun. 2020.
- Passos, A.M.A.; Aker, A.M.; Costa, R.S.C.; Santos, F.C.; Leite, V.P.D.; Marcolan, A.L. Effect of cover crops on physico-chemical attributes of soil in a short-term experiment in the southwestern Amazon region. *African Journal of Agricultural Research*, v.12, n.47, p.3339-3347, 2017. <https://doi.org/10.5897/AJAR2017.12800>.
- Pfüller, E.E.; Santos, D.B.; Aires, R.F.; Samaniego, M.D.P.G. Aspectos fenológicos e produtividade de espécies de verão para cobertura de solo em Vacaria, RS. *Investigación Agraria*, v.21, n.1, p.23-30, 2019. <https://doi.org/10.18004/investig.agrar.2019.junio.23-30>.

- Santos, H.G.; Jacomine, P.K.T.; Anjos, L.H.C.; Oliveira, V.A.; Lumbrreras, J.F.; Coelho, M.R.; Almeida, J.A.; Araújo Filho, J.C.; Oliveira, J.B.; Cunha, T.J.F. Sistema Brasileiro de Classificação de Solos. Brasília: Embrapa, 2018. 356p.
- São Miguel, A.S.D.C.; Pacheco, L.P.; Souza, E.D.; Silva, C.M.R.; Carvalho, Í.C. Cover crops in the weed management in soybean culture. *Planta Daninha*, v.36, p.e18172534, 2018. <https://doi.org/10.1590/S0100-83582018360100072>.
- Scavazza, A.L.; Soares, M.R.; Casagrande, J.C.; Medeiros, S.D.S.; Santi, P.H.P. Produção de fitomassa e extração de macronutrientes por plantas de cobertura cultivadas na estação seca. *Nativa*, v.6, n.6, p.619-624, 2018. <https://doi.org/10.31413/nativa.v6i6.5609>.
- Smith, H.F. An empirical law describing heterogeneity in the yields of agricultural crops. *Journal of Agricultural Science*, v.28, n.1, p.1-23, 1938. <https://doi.org/10.1017/S0021859600050516>.
- Sousa, D.C.; Medeiros, J.C.; Rosa, J.D.; Lacerda, J.J.J.; Mafra, Á.L.; Mendes, W.S. Chemical attributes of agricultural soil after the cultivation of cover crops. *Australian Journal of Crop Science*, v.11, n.11, p.1497-1503, 2017. <https://doi.org/10.21475/ajcs.17.11.11.pne799>.
- Sousa, R.P.; Silva, P.S.L.; Assis, J.P. Tamanho e forma de parcelas para experimentos com girassol. *Revista Ciência Agronômica*, v.47, n.4, p.683-690, 2016. <http://ccarevista.ufc.br/seer/index.php/ccarevista/article/view/4326>. 04 Jun. 2020.