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. Fora 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
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., 2017; Sousa et al., 2017), nematodes (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 soil 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., 2017, 2016) and sunnhemp (*Crotalaria juncea* L.) (Facco et al., 2017) by averages of the maximum curvature method of the coefficient of variation model (Paranaiba 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' 5", 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 dm⁻³; Mg: 1.5 cmol dm⁻³; Al: 0.3 cmol dm⁻³; H+Al: 8.7 cmol 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 dm⁻³; CTCpH7: 15.6 cmol 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 x 6 m (36 m²) was divided into 36 basic experimental units (BEU) of 1 x 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 BEU adjacent in the row and X BEU adjacent in the column were planned. The plots with distinct sizes and/or shapes were planned as (X=X₂×X₁), 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 X₁, X₂ and X₃ 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); Mᵢ - average of the plots with X BEU size; Vₙᵢ - variance among plots of X BEU size; CVᵢ - coefficient of variation (in %) among plots of X BEU size; and VUᵢ - variance per BEU among plots of X BEU size [VUᵢ=Vₙᵢ/Xᵢ].

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 VUᵢ=V₁/Xᵢ of Smith (1938) were estimated. These parameters were estimated by logarithmic transformation and linearization of the function VUᵢ=V₁/Xᵢ, i.e., log(VUᵢ)= log(V₁) - log(X), 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 \((Xo)\), in number of BEU, was calculated using the expression

\[
Xo = \frac{2(t_1 + t_2)^2 CV^2}{r d^2} \quad (\text{Hatheway, 1961}).
\]

In this expression \( b \) is the estimate of the heterogeneity index (in this study, the average \( b \) 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 \((\alpha = 5\% \) (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 \( t_1 = \text{INVT}(0.05; DF) \) and \( t_2 = \text{INVT}(0.40; DF) \), 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 (\( \text{Pennisetum glaucum} \) L), cultivar BRS 1501 (M) and showy rattlebox (\( \text{Crotalaria spectabilis} \) 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

| Composition | Trial \((1)\) | FM \((\text{g m}^{-2})\) \((2)\) | CV \((\%)(3)\) | \(b \) \((4)\) |
|-------------|----------------|-----------------|----------------|----------|
| 100\% M     | 1              | 6979            | 16.03          | 0.6828   |
|             | 2              | 6841            | 13.76          | 0.5453   |
|             | 3              | 7532            | 13.68          | 0.6995   |
|             | Average        | 7117            | 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            | 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            | 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            | 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            | 13.07          | 0.9629   |
| Overall     |                | 6994            | 14.62          | 0.9614   |

\(^{(1)}\) 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 ≤ b ≤ 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 (X₀), as a function of the number of repetitions.
Figure 1. Relationship between the variance per basic experimental unit (BEU) between X BEU plot sizes $[V_{U/X} = V_x/X^2]$, in thousands, and the planned plot size (X), in BEU, and the parameter estimates of the function $V_{U/X} = V_1/X^b$ of Smith (1938). Mass of fresh matter data obtained in uniformity trials, with 36 BEU of 1 m$^2$, formed by compositions of sowing densities of millet (*Pennisetum glaucum* L.), cultivar BRS 1501 (M), and showy rattlebox (*Crotalaria spectabilis*), common cultivar (CS).

Table 2. Optimum plot size, in m$^2$, 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  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 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 |
| 11 | 21.8 | 13.9 | 9.6 | 6.6 | 4.9 | 3.7 | 3.0 | 2.5 | 2.1 | 1.8 | 1.6 | 1.4 | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 |
| 12 | 19.9 | 12.0 | 8.3 | 6.0 | 4.3 | 3.0 | 2.5 | 2.1 | 1.8 | 1.6 | 1.4 | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 | 0.7 |
| 13 | 18.1 | 10.2 | 7.6 | 5.2 | 3.6 | 2.5 | 2.1 | 1.8 | 1.6 | 1.4 | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 | 0.7 | 0.6 |
| 14 | 16.3 | 8.5 | 6.0 | 4.0 | 2.8 | 2.1 | 1.8 | 1.6 | 1.4 | 1.2 | 1.1 | 1.0 | 0.9 | 0.8 | 0.7 | 0.6 | 0.5 |
| 15 | 14.9 | 6.9 | 4.5 | 3.0 | 2.1 | 1.6 | 1.4 | 1.2 | 1.0 | 0.9 | 0.8 | 0.7 | 0.6 | 0.5 | 0.4 | 0.3 | 0.2 |

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Continued from Table 2

| i  | r  | d (%) |
|----|----|-------|
| 4  | 5  | 6.2  |
| 5  | 6  | 6.0  |
| 6  | 7  | 5.8  |
| 7  | 8  | 5.6  |
| 8  | 9  | 5.4  |
| 9  | 10 | 5.2  |
| 10 | 11 | 5.0  |
| 11 | 12 | 4.8  |
| 12 | 13 | 4.6  |
| 13 | 14 | 4.4  |
| 14 | 15 | 4.2  |
| 15 | 16 | 4.0  |
| 16 | 17 | 3.8  |
| 17 | 18 | 3.6  |
| 18 | 19 | 3.4  |
| 19 | 20 | 3.2  |

- With fixed values of i and r, Xo 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, Xo decreased with the increase of r, and with fixed values of r and d, Xo 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, 181, 186, and 184 m², respectively (Table 3).

Other scenarios can be simulated using the expression

\[ X_o = \sqrt{2(b + t^2)} \times CV \times \sqrt{\frac{r\times d^2}{4}} \] (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; \( t_1 = \text{INVT}(0.05;32) = 2.03693334 \); \( t_2 = \text{INVT}(0.40;32) = 0.85299845 \); CV=14.62%; r=5; d=9%. Therefore,

\[ X_o = 0.9614 \times \sqrt{(2(0.9614 + 0.85299845)^2 \times 14.62^2 \times 9.9^2) = 9.6 \text{ BEU}.} \]

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; \( t_1 = \text{INVT}(0.05;28) = 2.048407115 \); \( t_2 = \text{INVT}(0.40;28) = 0.85464749 \); CV=14.62%; r=5; d=9%. Therefore,

\[ X_o = 0.9614 \times \sqrt{(2(0.9614 + 0.85464749)^2 \times 14.62^2 \times 9.9^2) = 9.7 \text{ BEU}.} \]

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., 2020).
### 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  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19  | 20  |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|-----|
| 20 | 20 | 52 | 329 | 253 | 163 | 128 | 969 | 778 | 638 | 533 | 451 | 387 | 335 | 293 | 258 | 229 | 205 |
| 15 | 30 | 508 | 319 | 218 | 158 | 123 | 903 | 744 | 669 | 554 | 455 | 379 | 321 | 273 | 238 | 208 | 184 | 163 |
| 10 | 15 | 499 | 314 | 213 | 151 | 118 | 925 | 743 | 609 | 509 | 431 | 369 | 320 | 280 | 246 | 219 | 196 | 176 |
| 5  | 10 | 437 | 304 | 212 | 154 | 117 | 914 | 734 | 602 | 502 | 425 | 364 | 316 | 276 | 243 | 216 | 193 | 174 |
| 3  | 5  | 480 | 304 | 214 | 151 | 116 | 914 | 734 | 602 | 502 | 425 | 364 | 316 | 276 | 243 | 216 | 193 | 174 |
| 2  | 5  | 485 | 305 | 208 | 151 | 114 | 898 | 721 | 592 | 494 | 418 | 358 | 310 | 271 | 239 | 212 | 190 | 171 |
| 2  | 3  | 482 | 303 | 207 | 150 | 114 | 892 | 717 | 588 | 490 | 415 | 356 | 308 | 270 | 238 | 211 | 189 | 169 |
| 2  | 2  | 479 | 303 | 206 | 149 | 113 | 887 | 712 | 584 | 488 | 413 | 354 | 307 | 268 | 236 | 210 | 187 | 168 |
| 2  | 1  | 467 | 302 | 202 | 152 | 119 | 963 | 790 | 659 | 558 | 478 | 414 | 362 | 319 | 284 | 253 | 228 |
| 2  | 1  | 458 | 301 | 202 | 119 | 936 | 749 | 599 | 492 | 435 | 364 | 316 | 276 | 243 | 216 | 193 | 174 | 158 |
| 2  | 1  | 446 | 300 | 200 | 128 | 138 | 969 | 744 | 669 | 554 | 455 | 379 | 321 | 273 | 238 | 208 | 184 | 163 |
| 2  | 1  | 437 | 299 | 200 | 118 | 138 | 969 | 744 | 669 | 554 | 455 | 379 | 321 | 273 | 238 | 208 | 184 | 163 |
| 2  | 1  | 429 | 299 | 200 | 118 | 138 | 969 | 744 | 669 | 554 | 455 | 379 | 321 | 273 | 238 | 208 | 184 | 163 |

### 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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