Sample Dimension for Estimation of Biomass and Yield of Sunn (Crotalaria juncea L.) and Showy rattlebox (C. spectabilis Roth)

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ABSTRACT
Green manure brings numerous benefits that promote, essentially, the maintenance and conservation of agro-systems and its implementation is fundamental to Brazilian Cerrado region. In this scenario, the present research aimed to determine the sample size for estimation biomass and productivity of sunn and showy rattlebox. The experiment was installed in the experimental area of the State University of Mato Grosso do Sul-Unit University Aquidauana (UEMS/UUA), located in the Brazilian Cerrado. It was randomly selected 45 plants in the experimental area of each crop to determine Fresh Mass (FM), Dry Mass (DM) and yield (YI), being the measures of central tendency, variability, asymmetry and kurtosis were calculated and checked for normality by Lilliefors’s test. In sunn and showy rattlebox, 340 and 197 plants, respectively, are sufficient for the estimation of evaluated descriptors, with confidence interval of 95%. The species evaluated did not differ for the characters FM and DM, both of which are recommended for cultivation in the Cerrado.

Key words: Experimental design, green manure, indirect selection

INTRODUCTION
The Cerrado occupation has occurred quickly, based on intensive production systems which have increased the processes of soil degradation. These processes result in impacts that often generate the inefficiency of agricultural systems, with frequent need for interventions through conservation practices to maintain productive potential of the soil. In this context, green manure becomes fundamental to the Cerrado region, because it promotes protection, improvement and maintenance of soil quality, as well as substantial increases in soil organic matter and nutrients, benefiting agroecosystems (Torres et al., 2014). Among the desirable characteristics for selection of species for green manure, stands out mainly dry mass production, because it is related to the capacity to increase nutrient by symbiosis with microorganisms, soil cover and recycling of nutrients (Calegari et al., 1993; Carvalho et al., 1999; Chaves and Calegari, 2001). Among the various botanical families species that can be grown as green manures, stand out those of the Fabaceae family. The legumes in addition to providing similar benefits to other species, have the ability to accumulate N through biological fixation (Melo, 2012; Da Silva et al., 2009; Leite et al., 2010).

Among the various legumes used as green manure, the species of the genus Crotalaria are very efficient as producers of biomass and as N fixing, being widely used in research (Silva et al., 1994; Carvalho and Amabile, 2006; Teodoro et al., 2011). However, so there is reliability of data obtained in a given study, it is important to consider the sample size to be measured to obtain an estimate of average with highest reliability. The sample size is directly proportional to data variability and to desired confidence degree in the estimate and inversely proportional to the estimation error allowed, initially established by the researcher (Barbetta et al., 2004; Bussab and Morettin, 2004; Spiegel et al., 2004).
Thus, the objective of this study was to determine the sample size for estimation biomass and productivity average in sunn and showy rattlebox and identify the most recommended species to cultivation in the Brazilian Cerrado.

MATERIAL AND METHODS

The experiment was installed in the experimental area of the State University of Mato Grosso do Sul-Unit University Aquidauana (UEMS/UAUA), in the Municipality of Aquidauana (MS), located in the Brazilian Cerrado (or Savanna), comprising the coordinates 20°27'S and 55°40'W, with an average elevation of 170 m.

The soil was classified as Ultisol sandy loam texture, with the following chemical characteristics in the layer 0-0.20 m: pH (H₂O) = 6.2; Al exchangeable (cmol, dm⁻³) = 0.0; Ca+Mg (cmol, dm⁻³) = 4.31; P (mg dm⁻³) = 41.3; K (cmol, dm⁻³) = 0.2; organic matter (g dm⁻³) = 19.7; V (%) = 45.0; m (%) = 0.0; sum of bases (cmol, dm⁻³) = 2.3 and Cation Exchange Capacity (CEC) (cmol, dm⁻³) = 5.1. The climate of the region according to the classification described by Köppen-Geiger is Aw (Savanna Tropical) with average annual rainfall of 1200 mm and maximum and minimum temperatures of 33 and 19°C, respectively (Torres et al., 2013).

Sunn and showy rattlebox were sown manually on April 16, 2013 in experimental areas with 50 m². The spacing used was 0.45 m between rows at a density of 15 plants m⁻¹ linear. No base fertilization and coverage for any culture were performed.

At maturation, it were randomly selected 45 plants in the experimental area of each crop to determine Fresh Mass (FM), Dry Mass (DM) and yield (YI). The FM was obtained through weighing of the plants on an analytical balance. After the plants were placed in paper bags and dried in forced circulation oven for 72 h at 65°C. The YI was obtained by weighing the grain in analytical balance and its moisture corrected to 13%. Measures of central tendency, variability, asymmetry, and kurtosis were calculated and checked for normality by Lilliefors’s test (Campos, 1983).

To each character, in each crop, the sample size was calculated (η), considering the 45 plants for a semi-amplitude of the confidence interval (estimation error) equal to 1, 2, ..., and 30% of the estimated average (a), with a confidence degree (1-α) of 95%, by the expression:

\[ \eta = \frac{t_{(1-\alpha/2)} \times s}{\varepsilon^2} \]

(Fonseca and Martins, 1995; Barbettá et al., 2004; Bussab and Morettin, 2004; Spiegel et al., 2004), where, \( t_{(1-\alpha/2)} \) is the critical value of Student t distribution, whose right area is equal to \( p(t>t_{(1-\alpha/2)}) = \alpha/2 \), with (n-1) liberty degrees, \( \alpha = 5\% \) of error probability and variance estimate (s²).

The statistical analysis was performed with assistance of the Genes computational program (Cruz, 2006) and Office Excel® software.

RESULTS AND DISCUSSION

The measures of central tendency, variability, asymmetry, kurtosis and Lilliefors’s test, in relation to characters FM, DM and YI in sunn and showy rattlebox, from 45 plants evaluated, data showed good adjustment to the distribution normal (p>0.05) (Table 1). However, according to the central limit theorem, even if the basic population is not normal, the distribution of the sample average will be approximately normal for samples greater than 30 observations (Fonseca and Martins, 1995; Bussab and Morettin, 2004). Given these considerations, in relation to normality can be inferred that the data from these characters provide credibility to the sizing of the sample size study by t-test.

Analyzing the standard deviation of both green manures verifies a superior magnitude of FM and DM on YI, suggesting higher sample sizes for the estimation of the fresh and dry mass relative to average productivity. Similar results were found in research on the sample design for evaluation of characters in other green manures (Burin et al., 2014; Cargnelutti Filho et al., 2014, 2015; Teodoro et al., 2014).

Moreover, the statistical parameters revealed existence of genetic variability among plants of sunn and showy rattlebox. These species did not differ how much biomass production (FM and DM), being within the limit recommended by Calegari et al. (1993), Darolt (1998), Carvalho et al. (1999) and Alvarenga et al. (2001) for cultivation in the Brazilian Cerrado region, furthermore sunn had a higher YI compared to showy rattlebox. It is important to mention that in front of observed results, these species of green manure provides greater dry mass production and consequently greater soil cover which entails numerous benefits such as increased weed suppression, greater soil humidity and smaller temperature oscillation, among others. In addition, sunn can be considered for family farms because of its high seed production and high prices in the market.

Sample size (plants number) to estimate the average of each character in sunn, with semi-amplitude of the confidence interval equal to 1% of average estimate and 95% confidence degree, ranged between 2,974 and 8,510 plants (Table 2), whereas, for the showy rattlebox this variation was 1,108-4,915 plants. Results in similar magnitude was obtained by Burin et al. (2014), Cargnelutti Filho et al. (2014), Teodoro et al. (2014) and Cargnelutti Filho et al. (2015) which too identified variability in sample size of others green manure species.

From a practical standpoint, it can be inferred that it is difficult to obtain average estimates of the descriptors evaluated in sunn and showy rattlebox, with estimation error of 1%, due to the large plants number to be measured. Does not fit in this study the judgment of maximum estimation error acceptable, getting the use this information to the researcher for his experimental design.

If the option is to obtain averages with 5% estimation error, taking into account the density of 15 plants m⁻¹, 0.45 m spacing and the evaluation these characters in 5 rows useful length of 5.0 m, plots with rows of 7 and 7.0 m length would be suitable for experiments with sunn, according to Fig. 1.
Table 1: Statistical parameters of the fresh mass, dry mass and yield of 45 plants of sunn and showy rattlebox. Aquidauana, MS, Brazil, 2013

| Parameters               | Sunn          | Showy rattlebox |
|--------------------------|---------------|-----------------|
|                          | FM (g plant⁻¹) | DM (g plant⁻¹) | YI (g plant⁻¹) | FM (g plant⁻¹) | DM (g plant⁻¹) | YI (g plant⁻¹) |
| Minimum                  | 60.88         | 24.06           | 3.30           | 55.03         | 31.29         | 3.20           |
| Maximum                  | 302.79        | 130.07          | 11.80          | 197.58        | 101.80        | 6.30           |
| Average                  | 136.28        | 61.39           | 7.28           | 102.67        | 61.24         | 4.45           |
| Variance                 | 3,891.00      | 520.35          | 3.88           | 1,275.54      | 336.19        | 0.54           |
| Standard deviation       | 62.38         | 22.81           | 1.97           | 35.71         | 18.34         | 0.73           |
| Asymmetry (1)            | 0.36          | 0.94            | 0.01           | 0.94          | 0.32          | 0.59           |
| Kurtosis + 3(2)          | 4.33          | 3.74            | 2.78           | 3.48          | 2.26          | 3.06           |
| p-value (3)              | 0.24          | 0.17            | 0.08           | 0.20          | 0.16          | 0.14           |

(1) Averages of each character measured in sunn and showy rattlebox, followed by the same letter do not differ by bilateral t-test at a significant level of 0.05, (2) Asymmetry differs from zero by t-test at a significant level of 0.05, (3) Not significant, (4) Kurtosis differs from three by t-test at 5% probability, p-value by Lilliefors' test at 5% probability, FM Fresh mass, DM: Dry mass and YI: Yield.

Table 2: Sample size to estimate the average of fresh mass, dry mass and yield of sunn and showy rattlebox for a interval semi-amplitude with 95% confidence and errors equal to 1, 2, …, 30% of the estimated average

| Errors (%) | Sunn | Showy rattlebox |
|------------|------|-----------------|
|            | FM   | DM   | YI   | FM   | DM   | YI   |
| 1          | 8,510| 5,608| 2,974| 4,915| 3,641| 1,108|
| 2          | 2,127| 1,402| 743  | 1,229| 910  | 277  |
| 3          | 946  | 623  | 330  | 546  | 405  | 123  |
| 4          | 532  | 351  | 186  | 307  | 228  | 69   |
| 5          | 340  | 224  | 119  | 197  | 146  | 44   |
| 6          | 236  | 156  | 83   | 137  | 101  | 31   |
| 7          | 174  | 114  | 61   | 100  | 74   | 23   |
| 8          | 133  | 88   | 46   | 77   | 57   | 17   |
| 9          | 105  | 69   | 37   | 61   | 45   | 14   |
| 10         | 85   | 56   | 30   | 49   | 36   | 11   |
| 11         | 70   | 46   | 25   | 41   | 30   | 9    |
| 12         | 59   | 39   | 21   | 34   | 25   | 8    |
| 13         | 50   | 33   | 18   | 29   | 22   | 7    |
| 14         | 43   | 29   | 15   | 25   | 19   | 6    |
| 15         | 38   | 25   | 13   | 22   | 16   | 5    |
| 16         | 33   | 22   | 12   | 19   | 14   | 4    |
| 17         | 29   | 19   | 10   | 17   | 13   | 4    |
| 18         | 26   | 17   | 9    | 15   | 11   | 3    |
| 19         | 24   | 16   | 8    | 14   | 10   | 3    |
| 20         | 21   | 14   | 7    | 12   | 9    | 3    |
| 21         | 19   | 13   | 7    | 11   | 8    | 3    |
| 22         | 18   | 12   | 6    | 10   | 8    | 2    |
| 23         | 16   | 11   | 6    | 9    | 7    | 2    |
| 24         | 15   | 10   | 5    | 9    | 6    | 2    |
| 25         | 14   | 9    | 5    | 8    | 6    | 2    |
| 26         | 13   | 8    | 4    | 7    | 5    | 2    |
| 27         | 12   | 8    | 4    | 7    | 5    | 2    |
| 28         | 11   | 7    | 4    | 6    | 5    | 1    |
| 29         | 10   | 7    | 4    | 6    | 4    | 1    |
| 30         | 9    | 6    | 3    | 5    | 4    | 1    |

FM: Fresh mass, DM: Dry mass and YI: Yield.

Fig. 1(a-b): Sample sized plots for average with 5% estimation error in (a) *Crotalaria juncea* and (b) *Crotalaria spectabilis*.
Considering these same variables for the showy rattlebox, plots of 5 rows with 7.0 m long would be suitable. However, according Teodoro et al. (2014), other factors must be analyzed for planning experiments with these green manures, such as seed germination, plant density, spacing to be used and type of harvesting and weeding (manual or mechanical).

CONCLUSION

In sunn and showy rattlebox, 340 and 197 plants, respectively, are sufficient for the estimation of evaluated descriptors, with confidence interval of 95%. The species evaluated did not differ for the characters FM and DM, both of which are recommended for cultivation in the Cerrado and similar regions of the world.

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