Short communication

Size and shape of the experimental unit in yield trials of *Brachiaria*, hybrid CIAT 3608

Jorge Claudio Vargas-Rojas https://orcid.org/0000-0002-1139-2148, Edgar Vidal Vega-Villalobos https://orcid.org/0000-0002-5678-1710 and Roberto Cerdas-Ramírez https://orcid.org/0000-0003-3415-1651

Universidad de Costa Rica, Sede Regional de Guanacaste, Liberia, Costa Rica. E-mail: jorgeclaudio.vargas@ucr.ac.cr

Abstract

Objective: To determine the size and shape of the experimental unit for yield trials of *Brachiaria,* hybrid CIAT 3608, in the Santa Cruz canton, Guanacaste, Costa Rica, through the application of the multiple regression method.

Materials and Methods: A uniformity trial was planted and the obtained data were used to apply the multiple regression method. According to it, the residual variability was measured as variation coefficient, corresponding to the different simulated sizes and shapes of experimental unit, and modeling was done depending on length and width. Two multiple regression models were adjusted: one of them included the term of interaction among the regressors, and the other did not.

Results: The model without the interaction term was the one with the best fit. It had higher R^2 and all the estimated coefficients were significant ($p < 0,05$). Then, the partial derivatives were calculated with regards to the length and width of the regression equation, estimated by the model without interaction, and they were equal to -1. The system of resulting equations was solved and the combination of width and length of the experimental unit, which minimizes the variation coefficient, was obtained. For this work, this combination turned out to be 4,95 m of length and the width is 6,03 m.

Conclusions: In the framework of the conditions under which this work was conducted, to obtain accurate results with the species *Brachiaria* hybrid CIAT 3608, one experimental unit of 5 x 6 m of length and width, respectively, is sufficient. That is, an experimental unit of 30 m^2.

Keywords: statistical analysis, mathematical model, experimental error, uniformity essay

Introduction

According to Gómez (1972), the experimental unit (plot) is the area in which the random allocation of the treatments in an essay is carried out. Rodríguez *et al.* (2018) define it as the basic information unit of experimentation. This information, which will become the results of the essay, comes from tests of probabilistic nature, for which it is linked not only to the performance that can be expressed by a certain set of treatments, but to other factors external to the essay. The latter cause extra variability, and tend to overshadow the effect of the treatments, called as a whole experimental error (Montgomery, 2017).

Ideally, the experimental units should be homogeneous, so that after the application of different treatments the difference (if any) can be ascribed to their effect exclusively, and not to other factors, which are known as "noise". Nevertheless, to obtain a set of totally homogeneous experimental units is impossible, particularly when trials are conducted in an agricultural field, due to the soil variability (Lohmor *et al.,* 2017).

The lack of homogeneity among the experimental units, not ascribable to known causes, generates a considerable effect on the magnitude of the data variability, that is, on the experimental error (Khan *et al.,* 2017). If this error is not controlled or quantified, its effect could distort the estimation of the means of the treatments and their comparison. Thus, if it is desired that the results of an essay are reliable, it is recommended to apply adequate experimental techniques, among which is the utilization of adequate size and shape of experimental unit (Condo and Pazmiño, 2015).

The specialized bibliography reports two reasons of primordial importance to justify the need to estimate an optimum size and shape of experimental

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unit (Rodríguez *et al.,* 1993; Sánchez-Hernández *et al.,* 2006). The first one is to reduce the experimental error of the essay (Guimarães *et al.,* 2019). The second one is based on economic and practical aspects: saving seed, space, economic resources and work. The plots with a lower size than the optimum can incur in unnecessary expenditures of time and resources (González *et al.,* 2018). Thus, the highest efficiency of an essay is supported on its optimum size and shape of experimental unit (Vallejo and Mendoza, 1992).

To determine the plot size and shape there are highly varied statistical methods. Most of them start from a uniformity trial. (Viloria *et al*., 2017). Álvarez-Torres *et al.* (1986) indicate that, although there are other methods, the uniformity trial is the most accurate, with the inconvenience that it requires a considerable investment of time and resources. The uniformity trial is a relatively large plot, treated in a uniform way, regarding fertilization, application of agrochemicals and other crop labors (without application of treatments), subdivided at the moment of harvest into small plots, called basic units (Schwertner *et al.,* 2015).

In the multiple regression model, one of the most widely used due to its efficiency, the basic units are grouped into plots of different shape and size. In these resulting plots the corresponding yield is quantified by adding the yields of the basic units. Then, for the total experimental units formed with a particular combination, the residual variability is estimated, measured as coefficient of variation (CV), related to the combination of the length and width of each experimental unit. This allows to model the CV according to the length and width, which generates a response surface in three dimensions. On such surface the changes in the CV can be studied, due to the length and width variations, and also their combination (length x width) can be obtained, which makes it possible that the surface has a certain curvature. Usually, the curvature that is sought is with the -1 slope, because after this point adding a new basic unit in the independent variable will result in a reduction of the dependent variable (CV) lower than the unit. Thus, the increase of size does not justify anymore the decrease in variability.

To minimize the obtained function with the multiple regression, the partial derivatives of the variation coefficient with regards to length and width should be calculated and both should be equal to -1, which allows to find the desired minimum. This method allows to find the size and shape of the plot, which minimizes the variability of the essay (Barrientos-García, 1981; Álvarez-Torres *et al.,* 1986).

The objective of this work was to determine the size and shape of the experimental unit for yield essays of *Brachiaria,* hybrid CIAT 3608, through the multiple regression method, in the Santa Cruz canton, Guanacaste, Costa Rica.

Materials and Methods

Location and climate. The essay was conducted during the period from August to November, 2019, in the Experimental Farm of Santa Cruz (N 10° 17’ 6,24’’ and W 85° 35’ 42,95’’), property of the University of Costa Rica, located in the Santa Cruz canton, Santa Cruz district, Guanacaste province, Costa Rica. This facility is located at 54 m.a.s.l. the average rainfall is 1 834 mm/year, with mean annual temperature of 27,9 ºC (Cerdas-Ramírez, 2017).

Experimental procedure. Planting was carried out in rows of 2 cm of depth, separated by 0,50 m. The applied planting method was drilling, with a sowing rate of 5-6 kg/ha of seeds from *Brachiaria*, hybrid CIAT 3608 (Mulato II). The management received by the plot was the same regarding fertilization, weeding, pests and diseases.

The uniformity trial technique, described by Rodríguez *et al.* (1993), was used. According to this method, a pasture plot of 12 x 12 m, that is, 144 m^2, was planted. Two meters of edge around its perimeter were left, and thus an area of 10 x 10 m (100 m^2) was obtained to conduct the uniformity trial. The soil was classified as Vertic Ustropept (Vargas and Navarro, 2019). This plot was selected because it represented the conditions of most of them, regarding topography and soil type.

Field measurements. Twenty days after planting, a grid was designed on the plot, for which bamboo stakes and ropes were used. Thus, the 100 microplots (basic units), of 1 m^2 each, were clearly identified. To each basic unit Cartesian coordinates were assigned, so that all of them were located and identified on the land. Both coordinates were given by distances in meters to Cartesian axes (X was the width, Y was the length of the plot). The forage harvest was carried out separately in each of the microplots 100 days after planting. All the pasture plants from each basic unit were cut at soil level and were placed in a sac previously identified with the number that corresponded to the harvested basic unit, according to the system of Cartesian coordinates. Afterwards, each of the sacs was weighed and the yield was obtained in kilograms.

Statistical analysis. With the entered data (production and Cartesian coordinates of each basic unit) each of the possible shapes and sizes of experimental units were conformed, obtained by the combination of the basic units, and the respective coefficient of variation (CV) was calculated. Afterwards, the CV was modeled depending on the length and width of the experimental units, according to equation 1. The assumptions of the model were tested with diagnostic plots (residual quantiles and residual plots *vs*. predicted values).

$$y_{ijk}=\beta_0+\beta_1\gamma_i+\beta_2\alpha_j+\beta_3\gamma_i^2+\beta_4\alpha_j^2+\beta_5(\gamma\alpha)_{ij}+\varepsilon_{ijk} \quad (1)$$

Where:

y_{ijk}= *k*-eth variation coefficient corresponding to the *i*-eth length and to the *j*-eth width.
γ_i= *i*-eth plot length. With *i*: 1,…,L.
α_j= *j*- eth plot width. With *i*: 1,…,A.
$(\gamma\alpha)_{ij}$= interaction of the *i*-eth length with the *j*-eth width.
ε_{ijk} = random error of the *k*-eth observation of the *i*-eth length and *j*-eth width.

For each of the estimated coefficient of the model of equation 1, the respective hypothesis test was done to establish whether the estimated value for each coefficient was different from zero or not. When the estimated coefficient was not significant ($p > 0{,}05$), it was excluded from the model. Then, the partial derivatives were calculated with regards to the length and width of the fitted function for the predicted value of the CV and were equaled to -1. All the procedures were carried out with the R language (R Core Team, 2017).

Results

In the second column of table 1 the estimations of each one of the model coefficients are shown, and in the fifth column the probability value associated to each term is found. It was observed that, with the exception of the interaction, all the terms were significant ($p < 0{,}05$), for which this term was excluded from the model of equation 1, and the model was fitted without interaction. The results are shown in table 2.

All the estimated coefficients of the model without interaction were significant ($p < 0{,}05$). In addition, this model had a R^2 of 0,92. Meanwhile, in the model with interaction, R^2 was 0,90. For such reasons, the model without interaction was selected to make the estimations.

The estimated regression function of the variation, related to the length and width, is presented in equation 2:

$$\widehat{CV} = 28{,}11\text{-}2{,}64L\text{-}4{,}85A+0{,}16L^2+0{,}31A^2 \quad (2)$$

Afterwards, the partial derivatives with regards to the length and width of equation 2 were calculated, and were equaled to -1. This resulted in the following system of equations:

$$\begin{bmatrix} 0{,}32L & 0 \\ 0 & 0{,}62A \end{bmatrix} = \begin{bmatrix} -1+2{,}64 \\ -1+4{,}85 \end{bmatrix} \quad (3)$$

The system of equation 3 was solved and the combination of length and width was obtained, in which the desired curvature point is achieved. This combination was 5,27 m of length and 6,16 m of width. For practical effects, it can be considered as an experimental unit 5 m long and 6 m wide, corresponding to an area of 30 m^2.

Discussion

The size that is defined here corresponds to the size of useful plot, and not to total plot. For such reason, to this size the edges that are considered necessary should be added, according to the specific conditions of the essay.

Table 1. Estimated regression coefficients and statistics, associated for the model with interaction.

Coefficient	Estimation	Standard error	t-value	P – value
Ordinate	28,88	2,31	12,51	< 5,00e-07
Length	-2,88	0,81	-3,54	< 0,01
Width	-5,08	0,81	-6,25	< 0,01
Length^2	0,17	0,06	2,59	0,03
Width^2	0,32	0,06	5,02	< 0,01
Length x width	0,04	0,07	0,54	0,60

Table 2. Estimated regression coefficients and statistics, associated for the model without interaction.

Coefficient	Estimation	Standard error	t-value	P – value
Ordinate	28,11	1,74	16,13	< 1,73e-08
Length	-2,64	0,66	-4,00	< 0,01
Width	-4,85	0,66	-7,35	< 0,01
Length^2	0,16	0,06	2,65	0,02
Width^2	0,31	0,06	5,31	< 0,01

Several studies have been conducted related to the sources of experimental error and the application of techniques to decrease such error (Lorentz *et al.,* 2010), in which it is mentioned that the most practical and accessible resource is the size of the experimental unit that will receive the treatment. However, frequently, the selection of the size of experimental unit when establishing an essay at field level is done based on arbitrary criteria (Álvarez-Torres *et al.,* 1986; Rodríguez *et al.*, 2018), which are not supported by scientific research. Barrientos (1981) and Oliveira *et al.* (2005) mention that the definition of the adequate size of experimental unit can be substantiated on empirical bases or on the researcher's experience and that, although these criteria are more or less valid, they cannot substitute the results derived from the different statistical methods to obtain an adequate size of experimental unit, because they are objective. Chacín (1977) states that another criterion to determine the size of experimental unit is the bibliographic review of works conducted in other localities, with the inconvenience that the adequate size of experimental unit is a regional characteristic, and is affected by the agroecological characteristics of the zone where the study was developed.

In published studies, related to Mulato II, the size of experimental unit varies from 4 to 1 200 m^2. This wide range of values can generate inconsistency and uncertainty in the researchers who take these studies as guide. In addition, this size has not been validated from the point of view of minimizing the experimental error. In spite of the importance of developing knowledge from local conditions, the research that has been conducted in this area is null.

At present, the studies about pastures aimed at forages in the tropic, such as Mulato II, are promising (Villalobos-Villalobos and Montiel-Longhi, 2015). This is due to the adaptive flexibility of this pasture type, which allows it an efficient growth, and to its high productions of good-quality biomass, even in soils with acidity and low fertility problems. Nevertheless, in the tropic, the soil is very variable in relatively small land spaces (Asif and Anver, 2003). Thus, soil heterogeneity will always be present in the trials conducted in the field, and it is one of the main causes of experimental error.

To minimize this error, besides other strategies, the appropriate size and shape for an experimental unit must be selected. However, although the size and shape of the experimental unit are conceived as a valuable tool to control the experimental error, their study has lost importance in recent years, which is reflected on the scarce bibliographic references from recent dates. This could be probably due to the boom of intensive cultivation technologies, in which large areas are subject to the treatments. Yet, in small countries, in which these production systems are not available, and there are limitations of land, and where the cultivation labors are still performed manually, this type of study is very helpful for local researchers, in order to maximize resources and obtain reliable results (Vargas and Navarro, 2019).

This work provides experimental information, which can be used as important tool when there are discussions about the size of the experimental unit for trials about the pasture Mulato II in agroecological zones similar to those of Santa Cruz, in Guanacaste. Thus, any technician or institution in charge of developing studies with this pasture can use the results of this work as starting point to define the size of the experimental unit.

The importance of this information is that it constitutes the result of a study conducted under local conditions. Utilizing these results to establish an adequate plot size would be more adequate than recurring to the revision of foreign literature or to arbitrary criteria. It is important to conduct this type of work in different agroecological zones, so that the way in which the size of the experimental unit changes, according to the environmental conditions, can be studied.

Conclusions

Under the conditions of this study, to obtain accurate results with the species *Brachiaria,* hybrid CIAT 3608, it is sufficient to establish an experimental unit of 5 x 6 m of length and width, respectively.

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Authors' contribution

- Jorge Claudio Vargas-Rojas: Project planning and conception, design of the uniformity trial, data collection and analysis, manuscript writing and correction.
- Edgar Vidal Vega-Villalobos: Project planning and conception, design of the uniformity trial, data collection and manuscript writing.
- Roberto Cerdas-Ramírez: Contributed to the project planning and conception and to the manuscript revision

Conflict of interests

The authors declare that there is no conflict of interests among them.

Bibliographic references

Álvarez-Torres, R. A.; Soto-Buriticá, Maria M. & Gómez-López, H. Tamaño de parcela y número de repeticiones para estimar una población de *Spodoptera frugiperda* (Smith) y su daño en maíz. *Rev. Fac. Nac. Agron*. 39 (2):5-16, 1986.

Asif, M. & Anver, M. Variability in fields experiments in maize crop in Pakistan. *Pak. J. Agric. Sci*. 40 (3-4):207-209, 2003.

Barrientos-García, M. *Evaluación de cuatro métodos para la determinación de tamaño y forma óptimos de parcela para experimentación agrícola*. Tesis presentada en opción al grado científico de Licenciado en Ciencias Agrícolas. San Carlos, Guatemala: Universidad de San Carlos, 1981.

Cerdas-Ramírez, R. Extracción de nutrientes y productividad de moringa (*Moringa oleifera*) con varias dosis de fertilización nitrogenada. *InterSedes*. 18 (38):145-163, 2017. DOI: https://doi.org/10.15517/isucr.v18i38.32673.

Chacín, F. Tamaño de parcela experimental y su forma. *Rev. Fac. Agron*. 9 (3):55-74, 1977.

Condo, P. L. & Pazmiño, G. J. *Diseño experimental en el desarrollo del conocimiento científico de las ciencias agropecuarias*. Riobamba, Ecuador: Escuela Superior Politécnica del Chimborazo, 2015.

Gomez, K. A. *Techniques for field experiments with rice: layout, sampling, sources of error*. Los Baños, Philippines: International Rice Research Institute, 1972.

González, G. G. H.; Morais, A. R. de; Caballero-Mendoza, C. A.; Bortolini, J. & Rodrigues-Liska, G. Estimación del tamaño óptimo de parcela en experimentación con batata dulce. *Agrociencia Uruguay*. 22 (2):7-12, 2018. DOI: http://dx.doi.org/10.31285/agro.22.2.2.

Guimarães, B. V. C.; Donato, S. L. R.; Aspiazú, I.; Azevedo, A. M. & Carvalho, A. J. de. Methods for estimating optimum plot size for 'Gigante' cactus pear. *J. Agric. Sci*. 11 (14):205-215, 2019. DOI: https://doi.org/10.5539/jas.v11n14p205.

Khan, M.; Hasija, R. C. & Tanwar, N. Optimum size and shape of plots based on data from a uniformity trial on Indian Mustard in Haryana. *MAUSAM*. 68 (1):67-74, 2017.

Lohmor, N.; Khan, M. & Kapoor, K. Estimation of optimum plot size and shape from a uniformity trial for field experiment with sunflower (*Helianthus annuus*) crop in soil of Hisar. *Int. J. Plant Soil Sci*. 15 (5):1-5, 2017. DOI: https://doi.org/10.9734/IJPSS/2017/31613.

Lorentz, L. H.; Boligon, Alexandra A.; Storck, L. & Lúcio, A. D. Plot size and experimental precision for sunflower production. *Sci. agric*. 67 (4):408-413, 2010. DOI: https://doi.org/10.1590/S0103-90162010000400005.

Montgomery, D. C. *Design and analysis of experiments*. Hoboken, USA: John Wiley & Sons, Inc, 2017.

Oliveira, S. J. R. de; Storck, L.; Lopes, S. J.; Lúcio, A. D.; Feijó, Sandra & Damo, H. P. Plot size and experimental unit relationship in exploratory experiments. *Sci. agric*. 62 (6):585-589, 2005. DOI: https://doi.org/10.1590/S0103-90162005000600012.

R Core Team. *R: A language and environment for statistical computing*. The R Foundation. https://www.R-project.org/, 2017.

Rodríguez, N.; Sánchez, H.; Cháves-Córdoba, B. & Pacheco, P. N. Determinación de tamaño y forma óptimos de parcela para ensayos de rendimiento con café. *Rev. colomb. estad*. 14 (27):50-64, 1993.

Rodríguez, R. A.; Nogueira, C.; Rosales, Roxanna; Silva, Patricia da & Moraes, Helena C. de. Tamaño óptimo de parcela y número de repeticiones para evaluar el rendimiento de boniato con mulch y suelo descubierto. *Agrociencia Uruguay*. 22 (1):90-97, 2018. DOI: http://dx.doi.org/10.31285/agro.22.1.9.

Sánchez-Hernández, M. A.; Mejía-Contreras, A.; Villanueva-Verduzco, C.; Sahagún-Castellanos, J.; Sánchez-Hernández, C. & Jiménez-Rojas, María C. Determinación del tamaño adecuado de parcela experimental en calabaza pipiana (*Cucurbita argyrosperma* hubervar. stenosperma). *Rev. Fitotec. Mex*. 29 (04):339-348, 2006.

Schwertner, D. V.; Lúcio, A. D. & Cargnelutt-Filho, A. Uniformity trial size in estimates of plot size in restrict areas. *Rev. Ciênc. Agron.* 46 (3):597-606, 2015. DOI: https://doi.org/10.5935/1806-6690.20150043.

Vallejo, R. L. & Mendoza, H. A. Plot technique studies on sweetpotato yield trials. *J. Amer. Soc. Hort. Sci*. 117 (3):508-511, 1992.

Vargas-Rojas, J. C. & Navarro-Flores, J. R. Tamaño y forma de unidad experimental para ensayos de rendimiento de arroz (*Oryza sativa*), en Guanacaste, Costa Rica. *UNED Research Journal*. 11 (3):355-360, 2019.

Villalobos-Villalobos, L. & Montiel-Longhi, Mayra. Características taxonómicas de pastos Brachiaria utilizados en Costa Rica. *Nutr. Anim. Trop*. 9 (1):39-56, 2015. DOI: https://doi.org/10.15517/nat.v9i1.19391.

Viloria, R.; Brito, Miriam; García, Judith & Garrido, M. J. Estimación del tamaño óptimo de parcela experimental en ají dulce (*Capsicum chinense* Jacq.). *Rev. Fac. Agron., UCV*. 43 (1):1-6, 2017.