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Article

Comparison of five methods for calculating the optimal size of the experimental plot with sugarcane

Luz Elena Barrantes-Aguilar[1]
Adrián González-Estrada[2]
Julio César Barrantes-Mora[3,§]

1 Universidad de Costa Rica. Montes de Oca, San José, Costa Rica. Tel. 506 15012060. (luz.barrantes@ucr.ac.cr). U

2 Campo Experimental Valle de México-INIFAP. Carretera los Reyes-Texcoco km 13.5, Texcoco, Estado de México. CP. 56250.

3 Liga Agrícola Industrial de la Caña de Azúcar (LAICA)-Departamento de Investigación y Extensión. Avenida 15, Calle 3, Goicochea, San José, Costa Rica. Tel. 506 23301000. (jbarrantes@laica.co.cr).

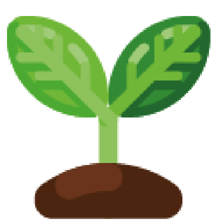
Autor para correspondencia: adrglez@prodigy.net.mx.

Abstract

Given the need to make efficient use of the resources allocated to experimental research in sugarcane crops, this research was proposed with the aim of determining the optimal size of the experimental plot in sugarcane crops in the Brunca region of Costa Rica. During the 2018-2019 harvest, an experimental uniformity trial was established, and five methods were compared: maximum curvature method, maximum curvature of the coefficient of variation, linear regression with constant, quadratic regression with constant and maximum distance method. The results indicate that the most efficient estimators were obtained with models that consider all sizes and forms of the uniformity trial (n=63). Segmented regression and linear regression with constant models produced the best estimators of the optimal size of the experimental plot: 72.16 and 93.22 m^2, respectively. With the other three methods, considerable and inconsistent differences in the sizes of the experimental plot were obtained. With the methods of maximum curvature and maximum curvature of the coefficient of variation, the results were so small: 14.01 and 12.5 m^2, respectively, that they are inadequate to carry out research in sugarcane; on the contrary, with the method of maximum distance, the size obtained was 157.48 m^2, statistically and economically inefficient. Therefore, the linear regression with constant and quadratic regression with constant models are appropriate for determining the experimental plot size in sugarcane. It was concluded that the recommended size to be used in the area is 72 m^2. This research was completed in December 2021.

Keywords:

estimators, experimental error, soil heterogeneity.



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Introduction

To achieve more efficient results in the design of experiments, Casler (2015) mentioned that repetitions, randomization, block definition, and experimental units should be analyzed; he also pointed out that the size of the experimental plot is the least understood aspect and the one with the least amount of theoretical and empirical results. According to Sripathi *et al*. (2017), the size of the experimental plot is one of the main causes of high residual variation in field experiments, which affects the statistical efficiency of the plot used as an experimental unit.

According to Smith (1938), there is a negative asymptotic relationship between variance by unit and plot size. For sugarcane, research has been carried out in Guatemala, such as the works of Palencia (1965); Álvarez (1982), in Brazil, that of Igue *et al*. (1991) and in India, that of Mahalanobis *et al*. (1939). Nonetheless, the validity of this research is local in nature.

Barrantes-Aguilar *et al*. (2020) estimated the optimal size of the experimental plot with sugarcane in the Brunca Region of Costa Rica with segmented regression models, but they did not apply other methods to compare their results and select the best one. The present research focused on determining the optimal size of the experimental plot in sugarcane crops of in the Brunca region of Costa Rica by comparing five methods to determine the most statistically efficient and best adapted to the conditions of the crop and the region where the study was carried out.

Materials and methods

The definition of the size of the experimental unit should be aimed at minimizing experimental error. In order to calculate the optimal size, uniformity trials have been used more frequently, in which a single plot of the same variety is sown, treated uniformly in terms of practices and crop management, the only factor that varies is the soil and its heterogeneity is one of the factors that most affect experimental error and efficiency (Sripathi *et al*., 2017).

The uniformity trial is divided into basic experimental units (BEUs), which are then grouped into different sizes and forms. Smith (1938) showed that there is a negative asymptotic relationship between variation and plot size, which he expressed as: $V_x=V^1/x^b$ 1) Where:V_x = corresponds to the variance of yield or any other variable of interest per unit area between plots of size x, V^1= is the variance between plots of a basic unit; x= the number of BEUs that make up the plot; and b is the soil heterogeneity index, where:0< b < 1.

With this coefficient and a cost ratio, Smith (1938) set out to find the optimal plot size as the area with which the greatest amount of information per unit of cost is obtained; that is,$x_0=bC_1/((1-b)C_2)$ 2). Where:C_1= is the portion of the total cost that is proportional to the number of plots per treatment; andC_2= the portion of the total cost that is proportional to the total area per treatment. Some methods have been proposed from the work of (Smith, 1938). The five methods that were evaluated in this research are explained below.

Maximum curvature method (MCM)

Lessman and Atkins (1963) explain the relationship between the coefficients of variation (CV_x) and the size of the plot with the following expression:CV_x= a/x^b 3). Where:

a and b

are obtained using the Gauss-Newton least squares method for nonlinear models; and x corresponds to the size of the plot.

From the first and second derivatives of (3), a curvature function is constructed; and from the first derivative of this function, the critical point (x_0) is obtained, which corresponds to the plot size with the highest rate of change of the coefficient of variation (Meier and Lessman, 1971):

$$x_0=\left[\hat{a}^2\hat{b}^2\left(2\hat{b}+1\right)/\left(\hat{b}+2\right)\right]^{1/\left(2+2\hat{b}\right)}$$

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4).

Where: $\hat{a}$ and $\hat{b}$

are estimators of a and b , respectively.

Method of maximum curvature of the coefficient of variation (MCCV)

This method has the advantage that it does not require grouping the basic units or adjusting a model, as in other cases (Sari and Lúcio, 2018). The method was proposed by Paranaíba *et al*. (2009a) and is based on the estimation of the first-order spatial autocorrelation coefficient between the basic experimental units:

$$\hat{\rho}=\sum_{i=2}^{fc}\left(\varepsilon_i-\dot{\varepsilon}\right)\left(\varepsilon_{i-1}-\dot{\varepsilon}\right)/\sum_{i=1}^{fc}\left(\varepsilon_i-\dot{\varepsilon}\right)$$

5).

Where: ε_i is the experimental error associated with each observation;; Z ε_{i-1} is the error of the previous observation; and $\dot{\varepsilon}$ is the average of the experimental error of all observations.

From this coefficient, the mean ($\tilde{Z}$) and variance (s^2) of the variable of interest measured in basic experimental units, the coefficient of variation for each size of plots (x) is obtained by using the following formula:

$$CV_x= 100\sqrt{(1-\hat{\rho}^2)s^2/\tilde{Z}^2}\Big/\sqrt{x}$$

6).

From the first and second derivatives of equation (6), the curvature function is obtained:

$$K=\frac{75\sqrt{(1-\hat{\rho}^2)}\,s}{\sqrt{\tilde{Z}^2}\,x^{2.5}\left(1+2500\frac{(1-\hat{\rho}^2)s^2}{x^3\tilde{Z}^2}\right)^{\frac{3}{2}}}$$

7).

The point where the curvature function is maximum is the optimal size:

$$x_0= 10\sqrt[3]{2(1-\hat{\rho}^2)s^2\tilde{Z}}\Big/\tilde{Z}$$

8).

This method has been considered suitable for obtaining the optimal plot size in rice, wheat, and cassava.

Linear regression with constant (LRP)

This model describes the relationship between the coefficient of variation and the plot size in two segments. The first is a line with a negative slope that decreases to a certain value and then behaves as a constant line. This model was proposed by Paranaíba *et al*. (2009a) and is mathematically represented as:

$$CV_x=\begin{cases}\beta_0+\beta_1x_i+\varepsilon_i,\ \text{if}:x\leq x_0;\\ CVP+\varepsilon_i,\ \text{if}:x>x_0.\end{cases}$$

9).

Where: CV_x = is the coefficient of variation between the totals for plots with x_i basic units; *CVP* = is the coefficient of variation at the point where the two segments meet; and ε_i = corresponds to the error associated with CVx, ε_i N $(0,\sigma^2)$. Due to the condition of continuity of the two segments that are equal at the point x_0 , we can write:

$x_0 = ((CVP-\beta_0))/\beta_1$

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10).

Quadratic regression with constant (QRP)

This model is represented by two segments: the first, described by a second-degree polynomial equation, and the second, by a constant line. As in the previous case, the optimal plot size is defined by the meeting point between the two segments. According to Moreira *et al*. (2016), the QRP model can be represented as:

$$CV_x=\begin{cases}\beta_0+\beta_1 x_i+\beta_2 x_i^2+\varepsilon_i,\ \text{if:} x\leq x_0;\\ CVP+\varepsilon_i,\ \text{if:} x>x_0.\end{cases}$$

11).

The function must be continuous and smooth, which means that the first derivatives with respect to *x* in both segments must be equal at the point x_0. Under this condition, the optimal size of the experimental plot is:

$x_0 = \beta_1/2\beta_2$

12).

Maximum distance method (MDM)

This method, proposed by Lorentz *et al*. (2012), seeks to solve the subjectivity of some methods, such as visual inspection, based on the geometry formed by: $y_c=a/x^b$ 13) and a line secant to the function (13), defined as: $y_R=cx+d$ 14). In (13), a and b can be obtained from the model of Smith (1938) or from Lessman and Atkins (1963). This method tries to find the point where the curve y_C is as far as possible from the line y_R, or in other words, it tries to find a line that maximizes that distance.

Uniformity trial as a source of information

The data used were from a uniformity trial that was established on May 29, 2018, at the El Porvenir farm, owned by CoopeAgri RL, which is located in La Fortuna de San Pedro in the canton of Pérez Zeledón, which belongs to the Brunca region, Costa Rica. Sugarcane of the RB 99-381 variety was sown in 40 rows 84 m long with a separation of 1.5 m.

In total there was 5 040 m^2 of experimental area, which was treated homogeneously in all the practices and tasks of the crop, once the edge was discounted there was 4 800 m^2 of useful plot. The basic experimental unit (BEU) was defined as 2 m long by 1.5 m wide (3 m^2), resulting in a total of 1 600 BEUs. The harvest and obtaining of weights of the trial were carried out manually on March 6 and 7, 2019. The variable of field yield was evaluated and the weight of each plot measured in kg was quantified, for which an electronic scale previously calibrated under the metric system was used.

For most of the methods, it was necessary to group adjacent BEUs, maximum curvature method (MCM), linear regression with constant (LRP), quadratic regression with constant (QRP) and maximum distance method (MDM), in different forms and sizes, considering that the entire experimental area should always be used. When tests of homogeneity of variances were necessary, an F-test was applied in the case of only two forms, and a Bartlett's test (Bartlett, 1937) was applied when three or more forms of grouping were involved.

In both cases, the null hypothesis of homogeneity of variances is tested. If the test was significant, the variance with the lowest value was taken to associate it with the corresponding size, if not, the variances were averaged. The statistical analysis of the information was carried out with the following packages: Microsoft Office Excel 2016, R version 4.2.1 (R Core Team, 2022), SAS version 9.3 (SAS Institute, 2011) and Python version 3 (Van-Rossum and Drake, 2009).

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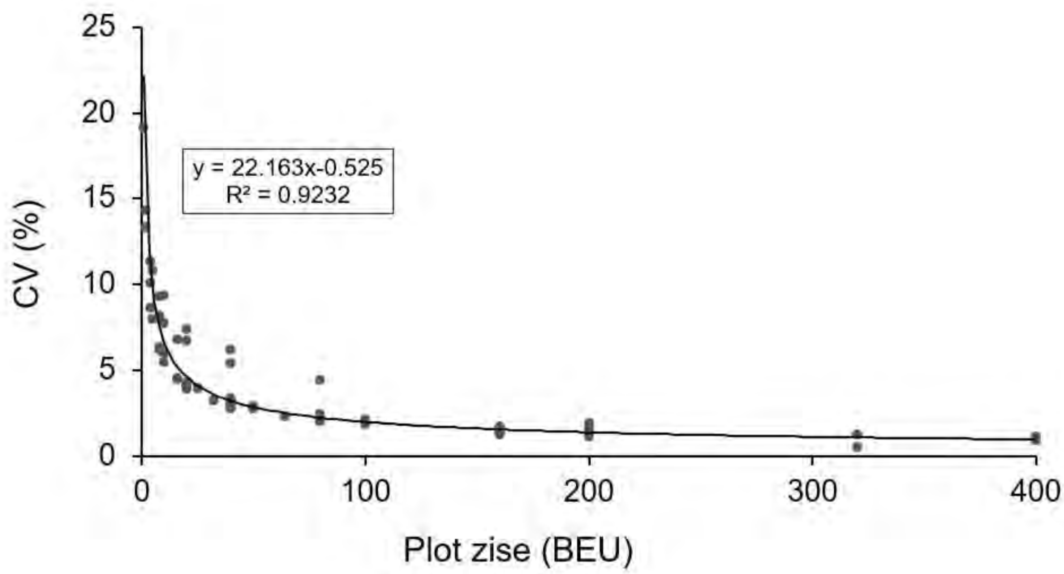
Results and discussion

We group data from 63 different forms, equivalent to 20 plot sizes, and considered only combinations that resulted in plot sizes that exactly fit the total area. For each form, the mean, sample variance and the coefficient of variation were calculated. As expected, the rate of decrease in the coefficient of variation decreases rapidly in the small plot segment but decreases more slowly for larger plots (Figure 1).

Figure 1. Relationship between the coefficient of variation (CV %) of sugarcane yield and plot size measured in BEUs.



Maximum curvature method (MCM)

To apply this method, it was necessary: first, to group the BEUs into different sizes and forms and second, to fit two models, one based on tests of homogeneity of variances and another that considers the cases in which, for the same size, there is more than one possible form of grouping (for example, the forms: 1x2 and 2x1represent 6 m^2 plots).

In the tests of homogeneity of variance, the smallest sizes always showed significant results (p< 0.05), so the variances obtained from clusters of the same size, but in different forms, were statistically different. From 96 m^2 onwards, only in some cases was the null hypothesis of equality of variances rejected. Once the variance was adjusted according to the results of these tests, the coefficient of variation associated with each size (CV_x) was obtained.

This information was used to fit model 1 (n=20). For model 2, we considered data from all forms and sizes, in this case n=63 (Table 1). Both models are based on equation (3) and their coefficients were significant (p< 0.01), with coefficients of determination greater than 90%. Using model one, the optimal size was 3.98 BEUs, equivalent to 11.94 m^2, and with model two the result was 4.67 BEUs, which means 14.01 m^2 plots. The smallest standard errors were obtained with model one.

Table 1. Results of the fit of models 1 and 2 by the maximum curvature method (MCM).

	Model 1		Model 2	
Constant	2.8843	**	3.0984	**
	(0.0334)		(0.077)	

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	Model 1		Model 2	
ln x	-0.492	**	-0.525	**
	(0.0085)		(0.0195)	
n	20		63	
F	3 344.27		721.32	
$R^2_{adjusted}$	99.43		92.07	
$\hat{a}$	17.89		22.16	
$\hat{b}$	0.49		0.53	
x_0	3.98		4.67	

Standard error in parentheses. Statistical significance at 5% (*) and 1% (**), respectively.

Method of maximum curvature of the coefficient of variation (MCCV)

The uniformity trial data were used to estimate the mean ($\tilde{Z}$), sample variance (s^2) and first-order spatial autocorrelation coefficient ($\hat{\rho}$). For the calculation of $\hat{\rho}$, the data were ordered in such a way that x_{i+1} was always the immediate plot closest to x_1, starting from north to south and then advancing from east to west, it is understood that the closest plants tend to influence each other more.

The coefficient of variation for each plot size (*x*) is:

$$CV_x = 100\sqrt{(1\text{-}0.10^2)14.48/19.91^2}\Big/\sqrt{x}$$

15).

With the first and second derivatives of (15), it is possible to obtain the values of the curvature function for each plot size (*x*). These values and the estimated coefficients of variation are substituted in equation (8). From the analysis of these results with the MCCV method, it was concluded that the optimal size of the experimental plot is 4.17 BEUs, equivalent to a 12.5 m^2 plot.

Linear and quadratic regressions with constant (LRP) (QRP)

Segmented regression models were adjusted for the scenario where variance homogeneity tests were applied (n=20) and for the case where all forms and sizes of the uniformity trial were used (n=63). The results are shown in Table 2 and the fit of the models in Figure 2. The coefficients were significant (*p*< 0.01) as was the F-statistic; the coefficients of determination ranged from 62 to 96%.

Table 2. Results of the fit of models 3, 4, 5 and 6 with LRP and QRP methods.

	LRP				QRP			
	Model 3		Model 4		Model 5		Model 6	
$\hat{\beta}_0$	16.7225	**	12.6093	**	19.3581	**	14.1172	**
	(1.3021)		(0.6728)		(1.5162)		(0.8055)	
$\hat{\beta}_1$	-1.3254	**	-0.4338	**	-2.589	**	-0.7654	**
	(0.2201)		(0.0537)		(0.5113)		(0.1149)	
$\hat{\beta}_2$					0.0991	**	0.0123	**
					(0.032)		(0.0031)	
n	20		63		20		63	
F	61.49		114.34		88.1		136.25	
$R^2_{ajusted}$	77.48		61.62		96.2		76.96	
P	2.35		2.17		2.44		2.23	
x_0	10.85		24.05		13.07		31.07	

Standard error in parentheses. Statistical significance at 5% (*) and 1% (**), respectively.

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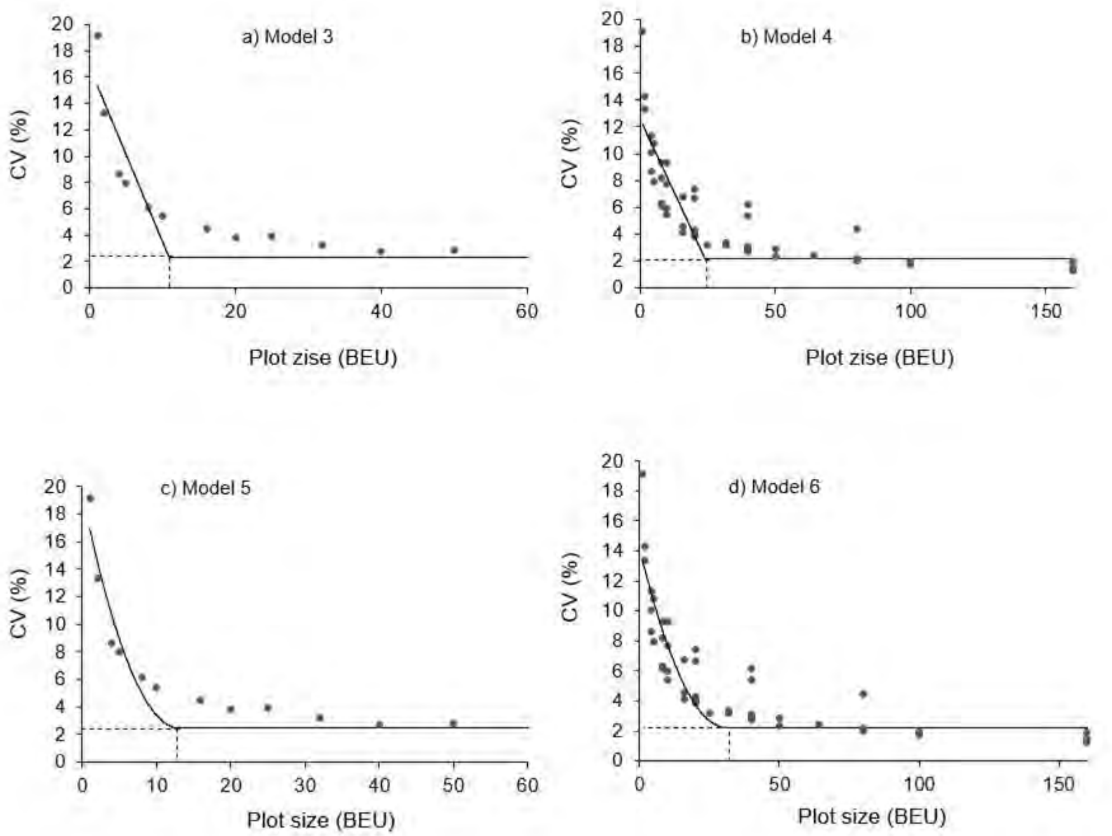
Figure 2. Relationship between the coefficient of variation (CV) and the plot size measured in BEUs and fit using the methods. a and b) LRP; c and d) QRP.



Plot size estimates were found to be between 10.85 BEUs ($\approx 32.54\ \mathrm{m}^2$) and 31.07 BEUs ($\approx 93.22\ \mathrm{m}^2$). Greater results were obtained with the application of the QRP model. Peixoto *et al*. (2011) used these segmented regression models in the estimation of plot size in *in vitro* preservation experiments of passion fruit (*Passiflora edulis*) and also found higher values when applying the QRP method than when calculating the LRP method, they attributed it to the curvature of the model. With both LRP and QRP, we obtained coefficients with lower standard error than when the 63 trial data were used.

Maximum distance method (MDM)

For the application of the MDM, the scenario in which the homogeneity of variance tests was applied (n=20) and in which they were not applied (n=63) were considered. For the values of a and b , those estimated by means of the MCM were used. As noted in Table 3, the plot size results obtained by this method differ greatly from the previous cases, with a size of approximately 56.21 and 52.49 BEUs estimated for models 7 and 8. These sizes represent plots of 168.63 and 157.48 m^2, respectively.

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Table 3. Results of the fit of models 7 and 8 by the maximum distance method (MDM).

Model	n	$\hat{a}$ †	$\hat{b}$ †	CV associated with a x_0	Size (x_0)
7	20	17.8908	0.492	2.4645	56.2109
8	63	22.1630	0.525	2.7433	52.4943

† a^ b^ = are estimated by using the MCM.

The summary of estimates for the different methods is shown in Table 4. For the MCM, the estimators were more efficient when the homogeneity of variance tests were performed (n=20); however, the difference with the other scenario was only 2 m^2, approximately.

Table 4. Plot size (m^2) with sugarcane estimated by the different methods.

Method		Size (m^2)	
		(n = 20)	(n = 63)
Maximum curvature method	MCM	11.9	14
Maximum curvature of the coefficient of variation	MCCV	-	12.5
Linear regression with constant	LRP	32.5	72.2
Quadratic regression with constant	QRP	39.2	93.2
Maximum distance method	MDM	168.6	157.5

Note that the MCCV method does not require grouping BEUs, so all 1 600 data points were used. The estimates by this method were very similar to those obtained by the MCM. The largest differences were found with MDM, which differ significantly from all others. And as intermediate results are the estimates by the LRP and QRP methods.

The maximum curvature method (MCM) of Lessman and Atkins (1963) is one of the most widely applied methods (Paranaíba *et al*., 2009b), although it tends to underestimate the size of the experimental unit. Silva *et al*. (2003) applied this and two other methods with eucalypt clones, they concluded that this method for the algebraic determination of the point of maximum curvature uses this and the apex of the curve, but not the point of stabilization of the values of the experimental coefficient of variation, which means that increases in the size of the experimental unit above the critical point (x_0) still bring significant gain to the experimental accuracy.

On the other hand, Henriques-Neto (2003), by using the MCM method, found small plot values that do not represent appropriate sizes for wheat research. Silva (2014) found differences in his estimates of up to 60% when comparing the MCM and the MDM, concluding that the estimation of the optimal plot size varies with the method used and with the characteristic evaluated. Henriques-Neto *et al*. (2009) came to a similar conclusion when evaluating yield characteristics of wheat grains. Cyprian *et al*. (2012) applied the MCM and evaluated eleven growth characteristics in coffee, they found that the plot size shows a different behavior according to the evaluated characteristic and the differences are up to 75%.

Leite *et al*. (2005) found plot sizes that were also not considered adequate, their estimates by MCM yielded results of less than one plant per plot for the estimation of genetic parameters in sugarcane families. Paranaíba *et al*. (2009b) compared four methods, including MCM, using experiments on

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wheat (four varieties) and cassava (two varieties and two traits). For wheat varieties, the smallest plot size was always the one estimated by the MCM, with differences of up to 83%. With cassava, the MCCV and MCM methods produced similar estimates, with differences between 1 and 3%.

MCM is not the only one with underestimation problems. In general, the results may vary depending on the method applied and it will be the responsibility of the researcher to select the one that best suits their situation. On the other hand, Paranaíba *et al*. (2009b) state that the value of the abscissa of maximum curvature should be interpreted as the minimum limit of plot size and not as the optimal size.

The minimum size of the experimental plot with sugarcane in the region is 72 m^2. This is the optimal size because, among all experimental plot sizes, it is the one that minimizes the coefficient of variation and because it has the smallest experimental cost among the other plots that have a coefficient of variation close to that of the LRP method with n= 63, but with a larger plot size and, consequently, with a higher experimental cost.

Conclusions

The results obtained by applying the five methods for estimating the optimal size of the experimental agricultural unit in sugarcane present very marked differences, of 145 m^2; the Brunca region uses plots with an approximate size of 80 m^2. The lack of feasibility in the application of the results of the MCM, MCCV and MDM methods indicates that they should be discarded. With the segmented regression models, values more in line with the conditions and characteristics of sugarcane crops in the region were obtained.

Those that yielded the most efficient estimators were those that considered all sizes and forms of the uniformity trial (n=63). It was concluded that the LRP and QRP models are appropriate for the determination of the size of the experimental plot in sugarcane in soils with conditions similar to those of the Brunca region of Costa Rica. The recommended size to use in the region is 72 m^2.

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