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Proposal of methods for determining the optimal size of experimental plots

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Abstract

Adequate planning of experiments is extremely important and for this to occur, the appropriate choice of plot size is essential. Thus, determining the plot size seeks to increase experimental precision, since precision decreases when a plot size smaller than the ideal is chosen; on the other hand, when opting for plot sizes larger than the ideal, the researcher may use more resources than necessary, as well as increasing the time to set up the experiment. Therefore, this work aimed to propose two new methods for determining the optimal size of experimental plots, which were applied in experiments with yellow passion fruit in the field, and compare them to the modified maximum curvature method. The maximum curvature method of the modified V_x function uses the equation proposed by Smith and the maximum curvature method of the modified CV_x function uses the equation proposed by Thomas. The modified maximum curvature method of the CV_x function proved to be suitable for estimating the optimal size of plots in experiments with yellow passion fruit. It is recommended to use the optimal plot size of 4 plants per plot for variables related to the fruit, 5 plants per plot for variables related to the pulp and for the production variables of 9 plants per plot.

Keywords: passion fruit; blank test; experimental precision; experimental planning.

1. Introduction

Adequate planning of experiments is extremely important and for this to occur, the appropriate choice of plot size is essential; since the researcher needs full knowledge of the experimental area in which the plot sizes, number of replications, and number of treatments will be combined, resulting in various levels of precision. Therefore, it is essential to have information about the available

experimental area, the variability of the material to be used, as well as the costs and labor that the experiment will require, and the appropriate analysis that evaluates the precision of the experiment (Cargnelutti Filho *et al.*, 2014).

Thus, determining the plot size seeks to increase experimental precision, since precision decreases when a plot size smaller than the ideal is chosen; on the other hand, when opting for plot sizes larger than the ideal, the researcher may use more resources than necessary, as well as increasing the time to set up the experiment (Galvão *et al.*, 2019), resulting in no gain in precision. It is worth mentioning that the ideal plot size also helps researchers in future experiments since the results obtained will serve as a basis for conducting new experiments, as well as a reference for experimental precision. That said, it is essential to use appropriate methods that determine the optimal plot size (Brito *et al.*, 2012).

Several studies, with different species, have been carried out in the field, greenhouse, and in vitro to estimate the optimal size of plots: coffee (Brioschi Junior *et al.*, 2020; Moraes *et al.*, 2019); tomato (Galvão *et al.*, 2019; Lúcio *et al.*, 2016); cucumber (Lúcio *et al.*, 2020); wheat (Cargnelutti Filho *et al.*, 2020); papaya (Celanti *et al.*, 2016; Faria *et al.*, 2020; Silva *et al.*, 2019b); banana (Silva *et al.*, 2019a); eucalyptus (Araújo *et al.*, 2015; Lopes *et al.*, 2020), are among the species studied.

Many methods are used to estimate the optimal size of plots, such as the maximum curvature method (Moreira *et al.*, 2016) with coffee cultivation; the modified maximum curvature method (González *et al.*, 2018; Guarçoni *et al.*, 2017; Michels *et al.*, 2020; Pires *et al.*, 2016), with soybean, coffee, sweet potato, and cabbage crops, respectively; segmented linear model method with plateau (González *et al.*, 2019, 2018; Guarçoni *et al.*, 2017; Moreira *et al.*, 2016) with sweet potato, radish, coffee, and cabbage, respectively; segmented quadratic model method with plateau (González *et al.*, 2018; Moreira *et al.*, 2016) with sweet potato and coffee, respectively; variance comparison method (Brioschi Junior *et al.*, 2020) with coffee; Hatheway method (Cargnelutti Filho *et al.*, 2020; Sousa *et al.*, 2016) with sunflower and wheat, respectively.

The most used method is the modified maximum curvature method, nonetheless, despite considering the variability of the experiment to determine the parameters, it is not taken into account when determining the optimal plot size. Analysis of the variability is necessary in any experiment, especially in field experiments where it becomes an essential part of the analysis. According to Grego & Vieira, 2005, field experiments are typically divided into relatively small plots or areas sampled at random; however, if experimental plots—even in small areas were considered to be uniform in terms of their attributes, the answers to the questions that already exist could be interpreted incorrectly because the hypothesis of the existence of spatial dependence would be unconsidered.

Therefore, this work aimed to propose two new methods for determining the optimal size of experimental plots, which were applied in experiments with yellow passion fruit in the field, and compare them to the modified maximum curvature method.

2. Materials and Methods

Two methods are proposed to determine the optimal plot size: the maximum curvature method of the modified V_x function and the maximum curvature method of the modified CV_x function, and were compared to the most currently used method of estimating the optimal plot size, the modified maximum curvature method.

The methods use blank test data (or uniformity test), in which plot sizes are simulated by grouping X_1 basic units in the row and X_2 basic units in the column, so that $X_1 X_2 = x$, represents x plot sizes, in basic units.

The analyzes were performed using the R software (R Core Team, 2020).

2.1 Maximum curvature method of the modified V_x function (MCVAR)

For the maximum curvature method of the modified V_x function, the function presented by Smith, 1938 was used:

$$V_x = \frac{V_1}{X^b} \tag{1}$$

where V_1 is the variance between plots composed of a basic unit and b is the regression coefficient that indicates the relationship between adjacent individuals.

It is determined that for the curve $y = V_x$, the radius of curvature is given by:

$$R_{V_x} = \frac{[1 + (V_x')^2]^{\frac{3}{2}}}{V_x''} \tag{2}$$

where V_x' and V_x'' , are the first and second derivative, respectively, of the function V_x .

To maximize the curvature, minimize R_{V_x} , that is, calculate the first derivative of the radius of curvature (equation 2). First, the logarithmic transformation is applied to R_{V_x} (equation 3):

$$\log R_{V_x} = \frac{3}{2} \log[1 + (V_x')^2] - \log V_x'' \tag{3}$$

and then, the first derivative of the V_x function is calculated (equation 4):

$$V_x' = \frac{-V_1 x^{-b} b}{x} \tag{4}$$

Afterwards, the second derivative of the V_x function is calculated (equation 5). Then:

$$V_x'' = \frac{V_1 x^{-b} b^2}{x^2} + \frac{V_1 x^{-b} b}{x^2} \tag{5}$$

Thus, substituting the derivatives (equations 4 and 5) in equation 3, we have:

$$\log R_{V_x} = \log \left(\frac{\left(\frac{V_1^2 x^{-2b} b^2}{x^2} + 1 \right)^{\frac{3}{2}}}{\frac{V_1 x^{-b} b^2}{x^2} + \frac{V_1 x^{-b} b}{x^2}} \right) \tag{6}$$

Therefore, the derivative of $\log R_{V_x}$ will be:

$$(\log R_{V_x})' = \frac{\left(-\frac{3V_1^2 x^{-2b} b^3}{x^3} - \frac{3V_1^2 x^{-2b} b^2}{x^3} \right) \left(\frac{V_1^2 x^{-2b} b^2}{x^2} + 1 \right)^{\frac{1}{2}} + \left(\frac{V_1^2 x^{-2b} b^2}{x^2} + 1 \right)^{\frac{3}{2}} \left(\frac{V_1 x^{-b} b^3}{x^3} + \frac{3V_1 x^{-b} b^2}{x^3} + \frac{2V_1 x^{-b} b}{x^3} \right)}{\frac{V_1 x^{-b} b^2}{x^2} + \frac{V_1 x^{-b} b}{x^2} \left(\frac{V_1 x^{-b} b^2}{x^2} + \frac{V_1 x^{-b} b}{x^2} \right)^2} \tag{7}$$

Equating $\log R_{V_x}'$ to zero, the value of the maximum curvature of the function V_x is obtained, given by:

$$X_c = \left[\frac{b^2 V_1^2 (2b + 1)}{(b + 2)} \right]^{\frac{1}{2b+2}} \tag{8}$$

where: X_c is the optimal plot size; V_1 is the variance between plots composed of a basic unit; b is the regression coefficient that indicates the relationship between adjacent individuals.

2.2 Maximum curvature method of the modified CV_x function (MCCV)

For the maximum curvature method of the modified CV_x function, the function presented by Thomas, 1974 was used, given by:

$$CV_x = \frac{V_1}{M_1 \sqrt{x^b}} \tag{9}$$

where: M_1 is the average of parcels composed of a basic unit; V_1 is the variance between plots composed of a basic unit; b is the regression coefficient that indicates the relationship between adjacent individuals. It is determined that for the curve $y = CV_x$ the radius of curvature is given by:

$$R_{CV} = \frac{[1 + CV_x'^2]^{\frac{3}{2}}}{CV_x''} \tag{10}$$

Analogous to the previous method, we use the logarithmic transformation in the radius of curvature (equation 11) as follows:

$$\log R_{CV} = \frac{3}{2} \log[1 + (CV_x')^2] - \log CV_x'' \tag{11}$$

and we calculate the first derivative of the function CV_x (equation 12):

$$CV_x' = -\frac{1}{2} \frac{V_1 b}{M_1 x (\sqrt{x^b})} \tag{12}$$

and the second derivative of the function CV_x (equation 13):

$$CV_x'' = \frac{1}{4} \frac{V_1 b^2}{M_1 x^2 (\sqrt{x^b})} + \frac{1}{2} \frac{V_1 b}{M_1 x^2 (\sqrt{x^b})} \tag{13}$$

Now, substituting the derivatives (equations 12) and (13) in equation 11, we have:

$$\log R_{CV} = \log \left(\frac{\left(1 + \frac{1}{4} \frac{V_1^2 b^2}{M_1^2 x^2 (x^b)}\right)^{\frac{3}{2}}}{\frac{1}{4} \frac{V_1 b^2}{M_1 x^2 (\sqrt{x^b})} + \frac{1}{2} \frac{V_1 b}{M_1 x^2 (\sqrt{x^b})}} \right) \tag{14}$$

Therefore, the derivative of $\log R_{CV}$ will be:

$$\begin{aligned} (\log R_{CV})' = & \frac{\left(\frac{\left(1 + \frac{V_1^2 b^2 x^{-b}}{4M_1^2 x^2}\right)^{\frac{3}{2}} \left(\frac{V_1 b^3}{8M_1 x^3 \sqrt{x^b}} + \frac{3V_1 b^2}{4M_1 x^3 \sqrt{x^b}} + \frac{V_1 b}{M_1 x^3 \sqrt{x^b}} \right)}{\left(\frac{V_1 b^2}{4M_1 x^2 \sqrt{x^b}} + \frac{V_1 b}{2M_1 x^2 \sqrt{x^b}} \right)^2} + \frac{\sqrt{1 + \frac{V_1^2 b^2 x^{-b}}{4M_1^2 x^2}} \left(-\frac{3V_1^2 b^3 x^{-b}}{8M_1^2 x^3} - \frac{3V_1^2 b^2 x^{-b}}{4M_1^2 x^3} \right)}{\frac{V_1 b^2}{4M_1 x^2 \sqrt{x^b}} + \frac{V_1 b}{2M_1 x^2 \sqrt{x^b}}} \right)}{\left(1 + \frac{V_1^2 b^2 x^{-b}}{4M_1^2 x^2}\right)^{\frac{3}{2}}} \times \\ & \times \frac{\left(\frac{V_1 b^2}{4M_1 x^2 \sqrt{x^b}} + \frac{V_1 b}{2M_1 x^2 \sqrt{x^b}} \right)}{\left(1 + \frac{V_1^2 b^2 x^{-b}}{4M_1^2 x^2}\right)^{\frac{3}{2}}} \end{aligned}$$

Equating $(\log R_{CV})'$ to zero, the value of the maximum curvature of the function CV_x is obtained, given by:

$$X_c = \left[\frac{b^2(b+1)V_1^2}{(2b+8)M_1^2} \right]^{\frac{1}{b+2}} \quad (15)$$

where: X_c is the optimal plot size; M_1 is the average of parcels composed of a basic unit; V_1 is the variance between plots composed of a basic unit; b is the regression coefficient that indicates the relationship between adjacent individuals.

2.3 Modified maximum curvature method (MCM)

The modified maximum curvature method was proposed by Meier & Lessman, 1971 and the exponential model, which will be used to estimate the optimal plot size considering the relationship between the coefficient of variation (CV) and the plot size with basic units, is given by

$$CV_x = \frac{a}{X^b} \quad (16)$$

where a and b are the parameters to be estimated. From the curvature function given by this model, the value of the abscissa at which the point of maximum curvature occurs was determined, as follows:

$$X_c = \left(\frac{a^2 b^2 (2b+1)}{b+2} \right)^{\frac{1}{2(b+1)}} \quad (17)$$

where X_c is the value of the abscissa at the point of maximum curvature, which corresponds to the estimate of the optimal size of the experimental plot. The determination coefficients were also calculated to verify the quality of the model fit for the different plot configurations.

2.4 Application on real data

The data used comes from a field experiment, with yellow passion fruit collected in 2008 at Embrapa Mandioca e Fruticultura. The experiment was conducted following a randomized block design, in which the blocks were three uniformity tests with the species. 100 plants were used, with each plant belonging to a single family; each of these plants was considered as a basic unit.

In the experiment, the plants were distributed in 10 rows by 10 columns and with 31 different shapes, 17 plot sizes were simulated, in which the number of plots varied from 100 to 2 and the plot size varied from 1 to 50 basic units per plot (Table 1).

Table 1. Number of plots (NP), plot size (PS) and plot shape (PSH) for the basic units of a field experiment with passion fruit in the field

PSH	NP	PS	PSH	NP	PS
1x1	100	1	5x2	10	10
2x1	50	2	3x4	6	12
1x2	50	2	4x3	6	12
3x1	30	3	3x5	6	15
1x3	30	3	5x3	6	15
2+1	25	3	4x4	4	16
1+2	25	3	3x6	3	18
2x2	25	4	6x3	3	18
2x2+1	15	5	4x5	4	20
2x3	15	6	5x4	4	20
3x2	15	6	5x5	4	25
2x3+1	10	7	5x6	2	30
3x2+1	9	7	6x5	2	30
2x4	10	8	5x10	2	50
4x2	10	8	10x5	2	50
2x5	10	10			

1x1, reads: one row value by one column value, 2x1, reads: two row values added in each column; 2+1, reads: add two row values adding one more column value; 2x2+1, reads: sum of two row values, two column values adding one more unit. Source: Authors.

The variables analyzed were the following: fruit length (FL, mm), fruit diameter (FD, mm), peel thickness (PT, mm), juice yield (JY, mL), soluble solids (Brix, °Bx), citric acid (Acidity, % citric acid), number of fruits (NF) and average fruit weight (FW, g).

3. Results and Discussion

In this section, the proposed methods Maximum curvature method of the modified V_x function and Maximum curvature method of the modified CV_x function were compared with each other (section 3.2) and among the most used method the Modified Maximum Curvature Method (section 3.1).

3.1 Modified maximum curvature method

According to Ferreira, 2018, the coefficient of determination evaluates how much of the data variability is described by the model, as well as verifying the quality of adjustment of the model used. Therefore, when adjusting the model, it was found that the values of the coefficient of determination (R^2) varied from 93%, for the Acidity variable, to 98%, for PT, Brix, and NF, all of which were considered good adjustments (Table 2).

Table 2. Estimates of model parameters using the modified maximum curvature method, coefficient of variation corresponding to the point of maximum curvature (CV (%)), value of the abscissa at which the point of maximum curvature occurs (X_c), coefficient of determination (R^2) for the variables under study in the uniformity test with yellow passion fruit

Variables	\hat{a}	\hat{b}	CV(%)	X_c	R^2
FL	5,10	0,65	3,27	1,97	0,97
FD	4,58	0,64	3,09	1,84	0,96
PT	16,14	0,52	8,08	3,78	0,98
JY	20,23	0,52	9,38	4,38	0,97
Brix	6,26	0,19	3,96	2,16	0,98
Acidity	10,29	0,36	7,58	2,33	0,93
NF	51,04	0,58	15,18	8,07	0,98
FW	14,12	0,52	7,38	3,48	0,95

FL: fruit length (mm); FD: fruit diameter (mm); PT: peel thickness (mm); JY: juice yield (mL); Brix: soluble solids (°Bx); Acidity: citric acid (% citric acid); NF: number of fruits; FW: average fruit weight (g).

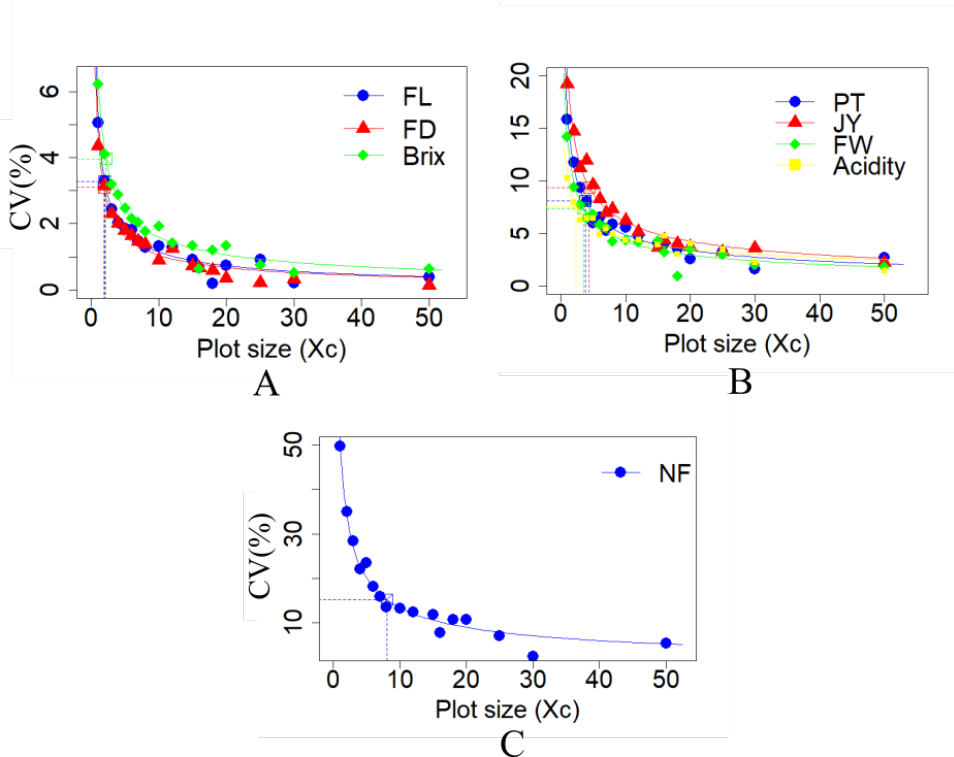


Figure 1. Relationship between the coefficient of variation (CV%) and plot size (X_c) in basic units for the variables under study in the uniformity test with yellow passion fruit using the modified maximum curvature method. (A) FL: fruit length (mm), FD: fruit diameter (mm), and Brix: soluble solids (°Bx); (B) PT: peel thickness (mm), JY: juice yield (mL), FW: average fruit weight (g) and Acidity: citric acid (% citric acid); (C) NF: number of fruits

Regarding the parameter estimates, it is possible to see that for the parameter \hat{a} , the estimates were quite variable, with a range equal to 46.46, which suggests that the variables show high divergence. As for the estimate of b , values closer to zero indicate that the experimental material is more homogeneous (low variability), and values closer to 1 tend to be heterogeneous (high variability). Therefore, it is noticed that the Brix and Acidity variables have low variability, and the

other variables have high variability. The values of the optimal plot sizes varied from 1.84 (DF), which corresponds to a point of maximum curvature of 3.09, to 8.07 (NF) corresponds to a point of maximum curvature of 15.18 (Table 2, Figure 1).

All optimal plot size values were always rounded to the next whole value to not lose any information. Therefore, for the optimal plot size to encompass all the variables studied using the modified maximum curvature method, 9 basic units (bu) are recommended (Table 2, Figure 1). Now, by group of characteristics of interest, we have that the optimal plot size for the variables related to the fruit (CF, DF, EC) is 4 bu, for the variables related to the pulp (JY, Brix, Acidity), it is 5 bu and production variables (NF, FW) the optimal plot size is 9 bu.

3.2 Maximum curvature method of the modified V_x function and Maximum curvature method of the modified CV_x function.

According to Lin & Binns, 1986, when b is greater than 0.7, it is preferable to use a smaller number of replications and a higher number of plots in each treatment to obtain appropriate experimental precision. From Table 3, it is noted that, in this study, b values ranged from 0.72 to 1.30 for the MCCV method and the MCVAR method, b values ranged from 0.69 to 1.27, where the majority was higher than 1 (in both methods) this suggests a low correlation, or even negative correlation, between neighboring plots, indicating competition between plants in the basic units (Thomas, 1974). According to Sousa *et al.*, 2016, it is possible to conclude that since the basic unit consists of just one plant, it would be possible to assume that next to each uncompetitive plant, there would supposedly be a more competitive one.

Furthermore, it is estimated that the adjustment of the two methods was good since the coefficient of determination varied from 93% to 98% for the MCCV method and from 97% to 99% for the MCVAR method. About parameter V_1 , there is an influence of other measurements on its calculation, which can be explained by the conversion between measurement units when divided or multiplied by a constant and also by the variance property that indicates that these values are further divided or multiplied by the squared constant (Table 3).

Table 3. Estimation of the parameters of the function $CV_{(x)}$ and the function $V_{(x)}$, estimates of the optimal plot size and corresponding values of the composite averages per basic unit (M_1) in the uniformity test with yellow passion fruit

Variables	MCCV					MCVAR			
	M_1	V_1	b	X_{cv}	R^2	V_1	b	X_{V_x}	R^2
FL	82.99	423.15	1.30	1.98	0.97	17.55	1.26	4.01	0.99
FD	76.82	352.06	1.28	1.84	0.96	11.42	1.13	3.36	0.99
PT	7.44	120.11	1.04	3.78	0.98	1.41	0.99	1.19	0.99
JY	493.83	9,988.01	1.04	4.37	0.97	9,262.55	0.92	110.63	0.98
Brix	14.20	88.94	1.19	2.16	0.97	0.78	1.16	0.96	0.99
Acidity	30.23	311.02	0.72	2.33	0.93	9.46	0.69	2.93	0.97
NF	64.84	3,309.34	1.16	8.07	0.98	1,335.50	1.27	26.81	0.99
FW	149.39	2,109.56	1.04	3.47	0.95	455.99	1.07	19.92	0.99

FL: fruit length (mm); FD: fruit diameter (mm); PT: peel thickness (mm); JY: juice yield (mL); Brix: soluble solids (°Bx); Acidity: citric acid (% citric acid); NF: number of fruits; FW: average fruit weight (g). X_{cv} and X_{V_x} are the optimal plot size for MCCV and MCVAR, respectively; M_1 is the average of parcels composed of a basic unit; V_1 is the variance between plots composed of a basic unit; b is the regression coefficient that indicates the relationship between adjacent individuals; R^2 is coefficient of determination

Therefore, in this work, it appears that the modified maximum curvature method of the function $CV_{(x)}$ estimates values of V_1 , in general, higher when compared to the maximum curvature method

of the $V_{(x)}$ modified; It is worth noting that MCCV estimates the values of V_1 weighting by the average M_1 .

Thus, it is possible to note that for MCVAR some estimated optimal plot sizes are very high, for experiments with passion fruit, given by the variable JY, with an optimal plot size of 111 bu, and by the variable NF, with an optimal size of 27 bu. Therefore, for these variables, the maximum curvature method of the modified $V_{(x)}$ function overestimates the optimal plot sizes, however, for the other variables the method proved to be suitable for estimating the optimal plot sizes. As for the MCCV method, it was found that it adequately estimated the optimal plot sizes, where the sizes varied from 2 to 9 basic units. Compared to the modified maximum curvature method, the optimal plot sizes estimated in the two methods are similar, both with good adjustments.

Thus, when suggesting an optimal size that considers all the variables in question, using the maximum curvature method of the modified $CV_{(x)}$ function, it would be 9 basic units. For the modified $V_{(x)}$ function method, 111 basic units would be needed per plot, or even calculating the average between the plot sizes would be needed 21 bu.

However, separating the variables by groups of characteristics of agronomic interest, one have that the optimal plot size for variables related to the fruit, such as CF, DF, and EC, is 4 bu, for variables related to pulp, JY, Brix and Acidity, the optimal plot size is 5 bu and for the production variables, NF and FW, it is 9 basic units in the maximum curvature method of the modified $CV_{(x)}$ function. For the maximum curvature method of the modified $V_{(x)}$ function, the optimal plot size for variables related to the fruit is 5 bu, for variables related to the pulp 111 bu, or even calculating the average, there is 39 bu; and for production variables, the optimal plot size is 27 bu or calculating the average, one have 24 bu.

Compared to the modified maximum curvature method with the modified maximum curvature method of the function $CV_{(x)}$, it is possible to observe a similarity between the optimal plot sizes estimated in the two methods, in which both demonstrated great adjustment. Therefore, the MCCV method can be used to obtain the optimal size of plots as it demonstrates practicality. Furthermore, it is a model that does not depend on personal criteria or quality of adjustment, even if the measure of variance between plots composed of a basic unit has been added.

The modified maximum curvature method (MCM), according to Paludo *et al.*, 2015, estimates smaller plot sizes than other methods, however, with a higher R^2 , such as those obtained in this study with yellow and purple passion fruit. According to Facco *et al.*, 2018, this method also allows the estimation of intermediate plot sizes in contrast to predetermined plot sizes, such as the variance comparison method. The maximum curvature method of the modified $CV_{(x)}$ function (MCCV) proved to be suitable for determining the optimal plot size, with good adjustment, in which the estimated values for the optimal plot size are similar to those estimated by the MCM method, considering the two species studied. The modified maximum curvature method of the $V_{(x)}$ function, despite being well adjusted, overestimated the optimal plot size for some variables, for the two species studied; was regarded as unstable since it varied according to the size of the uniformity test's fundamental unit or the unit of measurement used to assess the characteristic.

Thus, the modified maximum curvature and maximum curvature of the modified $CV_{(x)}$ function methods presented the regression models with the best adjustments, where the coefficient of determination (R^2) varied from 93% to 98% (Tables 2 and 3).

4. Conclusion

In the experiment with the species *Passiflora edulis* Sims (yellow passion fruit), the optimal plot sizes varied according to each method. The modified maximum curvature method of the $CV_{(x)}$ function proved to be suitable for estimating the optimal size of plots in experiments with yellow

passion fruit.

It is recommended to use the optimal plot size of 4 plants per plot for variables related to the fruit (fruit length, fruit diameter, and peel thickness), of 5 plants per plot for variables related to the pulp (juice yield, Brix and Acidity) and for the production variables (number of fruits and average fruit weight) the optimal size of 9 plants per plot is suggested.

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Conflicts of Interest

The authors declare no conflict of interest.

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