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# Optimal plot size in the experiment with low-cold demanding apple trees

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## ABSTRACT

Apple cultivation in the tropical conditions in northern Espírito Santo state is recent and, to increase the cropped area, basic field experimentation research is still necessary. Thus, the objective of this work was to determine the optimal plot size (OPS) for experiments with apple plants that are not very demanding in cold conditions of the cultivars Eva, Julieta, and Princesa, based on the evaluation data of three characteristics, in northern Espirito Santo state. After three years and three months, the stem diameter, production in the number of fruits per plant, and mass production per plant were evaluated. To determine the OPS, two methods were used, the maximum curvature modified in its original form, and the maximum curvature modified with bootstrap simulation, both according to Meier and Lessman. The two methods presented similar results for each characteristic, however the productive characteristics presented higher OPS compared to the vegetative characteristic of stem diameter. When setting up field experiments with apple plants of the Eva, Julieta, and Princesa varieties in conditions in the north of the state of Espírito Santo, it is recommended to plan with six plants in each experimental plot.

**Keywords:** bootstrap; experimental design; *Malus domestica* Borkh; simulation;

## INTRODUCTION

Apple tree cultivation on a commercial scale in Brazil began in the 1970s, where Golden Delicious, Starkrimson, Blackjon, and Melrose were the first varieties planted, but soon replaced by 'Gala' and 'Fuji' [1]. The progress in apple tree improvement works has allowed the launch of several cultivars that are not very demanding in cold conditions and their cultivation has been an adequate option due to the diversification of agricultural crops in the tropics, as seen in recent years in Brazil.[2, 3, 4]

Among the cultivars that stand out in Brazil for their productivity and fruit quality are Eva, Princesa and Julieta. [5] However, work on these cultivars is required to better understand their productive behavior over the years[6, 7] and in different growing regions due to the possible interaction between genotypes and environments.

One of the less demanding varieties at low temperatures, as highlighted by Lopes et al[6], the Eva cultivar stands out for producing sweet, low-acid fruits, with orange-red skin and conical shape. In the case of the Julieta cultivar, its fruits have a sweet, slightly acidic flavor, weighing more than 150 grams. The Princesa cultivar stands out for its firm pulp, crunchy appearance, slightly acidic flavor, bright red color, and medium-sized fruits.[6]

To boost the expansion of cropped areas for cultivars that are not very demanding in cold conditions, it is crucial to carry out well-planned experimental evaluations. In the planning process, after defining the design to be adopted and the characteristics to be studied, researchers move on to quantify the material necessary to carry out a quality experiment and, for this, it is essential to determine the size of each plot.

It was not possible to find scientific works that indicate the plot size to be adopted in experiments with apple trees that are not very demanding in cold conditions. Thus, experimental research has been carried out with different plot sizes ranging from one plant per plot[8], two plants per plot[9], three plants per plot[3, 10] up to 10 plants per plot[4].

To reduce experimental error, the researchers increase the size of the plot, which may result in unnecessary expenditure on experimental materials and physical space. The optimal plot size is then sought to improve experimental precision and optimize the acquired information, in addition to supporting further experiments.[11, 12, 13]

Regarding the methods for determining the optimal plot size, the literature presents several methodologies, the most currently used being the maximum modified curvature[11] and the maximum curvature of the coefficient of variation[12]. More recently, Celanti et al[13] proposed the use of bootstrap simulations to determine the optimal plot size, which, according to the authors, has the great advantage of a more rapid analysis as it is not necessary to identify the position of the plant in the test areas in white, which was also confirmed by Santos et al.[14]

Given the advancement of agricultural frontiers and the beginning of the cultivation of apple trees that are not very demanding in the cold in northern Espírito Santo state, the objective of this work was to determine the optimal plot size, using the modified maximum curvature method[11] with bootstrap simulation.[13]

## MATERIAL AND METHODS

The work was carried out with apple trees (*Malus domestica* Borkh) of the cultivars Eva, Julieta, and Princesa, and the collecting samples were from commercial crop plants on Boa Vista farm, located in the municipality of Montanha, northern Espírito Santo state in Brazil, located at the geographical coordinates 18° 8'8.63"S and 40°13'52.64" W (Figure 1). The region's climate, according to Koppen's classification, is the Aw-type, with dry winters and rainy summers.[15]

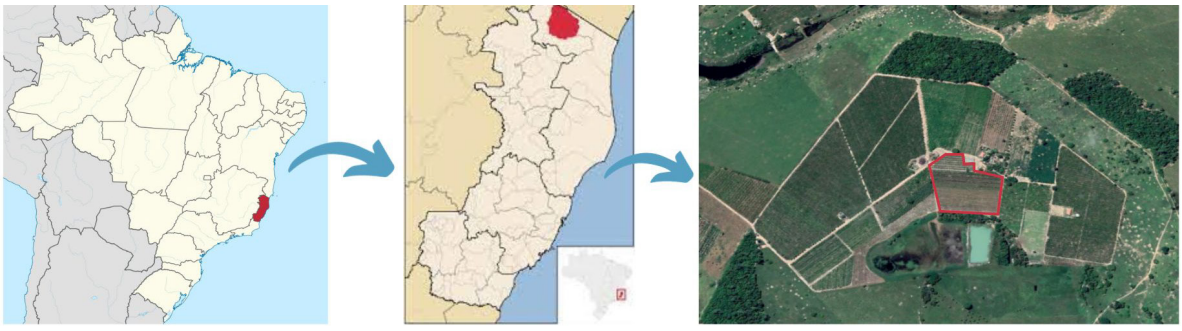

**Figure 1**: Map with the location of the apple (*Malus domestica* Borkh) orchard in the municipality of Montanha – ES.

The crop was implemented on September 5, 2014, with a spacing of 1.5 x 4.0 m, totaling 6 $m^2$ $plant^{-1}$, with a density of 1667 plants $ha^{-1}$. Planting was carried out with alternating rows between cultivars, following the recommendation of planting at least two compatible apple cultivars per area[3, 16, 4] considering that the apple tree is self-incompatible.[17]

All cultivars were from clonal seedlings using 'Marubakaido' as rootstock, with bare seedlings acquired from an accredited nursery in southern Brazil. The irrigation adopted was through drip with Microjet, 2 L $h^{-1}$, and the base fertilization was done with the agricultural fertilizer Minho Fertil®, 3 t $ha^{-1}$, in March and October, next to the plant.

To standardize flowering, in August 2017, the plants were defoliated using 1% copper sulfate with 3% urea, and, after defoliation, hydrogenated cyanamide and mineral oil were applied, following a recommendation by Lopes et al.[6]

One hundred plants of each cultivar were evaluated in a blank test. So, the 100 central plants were used from a total of 130 plants in a row of each cultivar, as shown in Figure 2.

In November 2017, fruit production per plant (NFR) was evaluated by counting fruits with commercial standards. In December 2017, the stem diameter (SD, in cm, measured 30 cm from the ground) and production per plant (PPP, in kg) were evaluated when the crop was three years and three months old. For this last evaluation, 10 fruits were randomly harvested from each plant of each variety, determining the average fruit mass (MFR) and then PPP = NFR x MFR.

Before determining the optimal plot size, a descriptive statistical analysis of each characteristic was carried out for the 100 plants of each cultivar. The following were obtained: mean ($\hat{m}$); minimum value (min); maximum value (max); standard deviation (s); coefficient of variation (CV).

The determination of the optimal plot size was made for each of the three characteristics using two methodologies: the modified maximum curvature, according to Meier and Lessman[11], represented by ML; maximum modified curvature, according to Meier and Lessman[11] using a resampling bootstrap simulation with 2000 replacements, according to Celanti et al[13], represented by MLboot.

For the ML methodology, it is necessary to establish the neighborhood relationship between the plants evaluated. For this, groupings were made for different plot sizes, as shown in Table 1.

Data analysis for each variable for each designed plot size generates coefficient of variation ($CV_i$) values. The relationship between the CV values and the respective different portion sizes ($X_i$) allows the generation of the power model equation according to equation 1, where the values of $\hat{\beta}_0$ and $\hat{\beta}_1$ are estimated through the logarithmic transformation of the power function.

$$CV_i = \hat{\beta}_0 X_i^{-\hat{\beta}_1} \quad (1)$$

Meier and Lessman[11] estimated the optimal plot size for the maximum curvature ($X_{oML}$) according to Equation 2.

$$X_{0ML} = \left[\hat{\beta}_0^2\hat{\beta}_1^2\left(2\hat{\beta}_1 + 1\right)/\hat{\beta}_1 + 2\right]^{\left(1/2+2\hat{\beta}_1\right)} \quad (2)$$

The optimal plot size was graphically represented together with the relationship between the values of the coefficient of variation and plot sizes.

To verify the correlation between contiguous plots, the heterogeneity index (b) was determined according to Smith[18], given by Equation 3, according to Lorentz, Erichsen e Lúcio[19], with b values closer to zero indicating high correlation and values closer to one indicating low correlation.

$$b = 2\hat{\beta}_1 \quad (3)$$

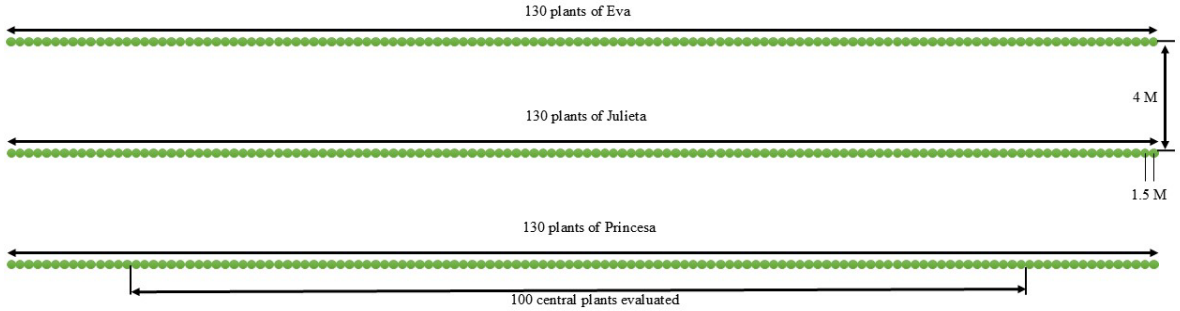


**Figure 2**: Illustration of the arrangement, in the field, of apple plants (*Malus domestica* Borkh) of the cultivars Eva, Julieta, and Princesa.

**Table 1:** Representation of the eight clusters formed according to the plot size designed in basic experimental units of a plant, and the corresponding shape and number of plots

| Groupings | Plot size ($X_i$) | Parcel size | Number of Size |
|---|---|---|---|
| 1 | 1 | 1 x 1 | 100 |
| 2 | 2 | 1 x 2 | 50 |
| 3 | 4 | 1 x 4 | 25 |
| 4 | 5 | 1 x 5 | 20 |
| 5 | 10 | 1 x 10 | 10 |
| 6 | 20 | 1 x 20 | 5 |
| 7 | 25 | 1 x 25 | 4 |
| 8 | 50 | 1 x 50 | 2 |

For Meier and Lessman' method[11] using a bootstrap resampling simulation, according to Celanti et al[13], MLboot, there is no neighborhood relationship because the values that make up each plot size ($X_i$) are obtained randomly among the available values, in this case, 100 values for each variable of each variety. Equations 1, 2, and 3 are equally applicable to bootstrap simulation data.

From the estimates $\hat{\beta}_0$ e $\hat{\beta}_1$ obtained from equation 1 and, taking into account the optimal plot size ($X_0$) found by equation 2, the coefficient of variation corresponding to the optimal plot size ($CV_{(X0)}$), according to equation 4, for each of the three characteristics of the three varieties.

$$CV_{X_0} = \hat{\beta}_0 X_0^{-\hat{\beta}_1} \quad (4)$$

The optimal plot size was adopted in the experiment, with an apple tree that is not very demanding on cold in the north of Espírito Santo, the largest size found among the three characteristics of the three varieties obtained by both methods.

To verify the applicability of the optimal plot size, the mean test according to Tukey was carried out, admitting the comparative evaluation of the three varieties for the three characteristics under study in a completely randomized design with seven replications, a number necessary to fulfill the minimum number of experimental plots according to the guidelines of Pimentel-Gomes[20]. The values of the mean square of residues (QMRes) necessary for the analysis were obtained according to equation 5, where $CV_{x0}$ is the largest among the varieties for the characteristic under analysis and $\hat{m}_i$ represents the average of the three varieties for the characteristic.

$$QMRes = \left[\left(CV_{X_0}\right)\left(\hat{m}_i\right)\right]^2 \quad (5)$$

The comparative evaluation among the three varieties for each of the three characteristics was also carried out in the case of using only one plant per plot and in the case of using all 100 plants from the blank trial considering one plant per plot.

The analyses and graphs were carried out using the R language[21] and, in the case of bootstrap simulation, procedures were used according to the script published by Celanti et al[13]. As it is a discrete random variable, the optimal size of the final installment was presented as a whole number, rounding to the nearest whole number.

## RESULTS AND DISCUSSION

Figure 3 illustrates the apple plants that are not very demanding in cold conditions 'Eva', 'Julieta', and 'Princesa', at approximately three years old, in commercial farming.

The descriptive statistics analyzed by mean ($\hat{m}$), minimum (min), maximum (max), standard deviation (s), and coefficient of variation (CV) are found in Table 2. The stem diameter was less than 10 cm for the three varieties. The average number of fruits per plant and mass per plant was higher in the Eva and Julieta varieties compared to the Princesa variety. The methodology of Meier and Lessman[11] for optimal plot size is not influenced by the average but rather by the variability measured by the CV and the soil heterogeneity index (b) by Smith.[18] Therefore, it is expected that the smallest optimal plot size will be for stem diameter that presents a CV ≅ of 10%, and the largest optimal plot size will be for the two traits that measure productivity per plant with CV>24%.

The statistics of Equations 1, 2, 3, and 4, obtained from the initial relationship among the different plot sizes (Table 1), and the respective CV values are found in Table 3. Smith's[18] heterogeneity index b is important in Meier and Lessman[11] plot size determination. Although the index

b does not enter directly into the Meier and Lessman[11] equation, it is demonstrated that $b = 2\hat{\beta}_1$ [19], with $\hat{\beta}$ being the estimate of a parameter from equation 1, also present in equation 2, which estimates the optimal plot size according to Meier and Lessman.[11]

The b index is a good indicator of the relationship between neighboring plants as the b value close to zero indicates homogeneous conditions between plants, for the characteristic under analysis, and values closer to unity indicate heterogeneity among neighboring plants. In this work, the b values, using the original method of Meier and Lessman[11], were close to unity in most determinations, therefore indicating that there is considerable heterogeneity between neighboring plants. Thus, the use of the b index obtained through bootstrap simulation according to Celanti et al[13] is advisable for working with apple plants that are not very demanding in cold conditions in the north of the state of Espírito Santo, considering that the values are equivalent to unity and, in practice, it becomes less costly as it eliminates the need for grouping the plants since in the simulation, the resampling is random and with replacement.


**Figure 3**: Photographs of the different apple tree cultivars in the orchard: cultivar Eva; cultivar Julieta; cultivar Princesa.

**Table 2:** Mean ($\hat{m}$), minimum value (min), maximum value (max), standard deviation (s), and coefficient of variation (CV) for three characteristics in three apple tree varieties that are not very demanding in cold, evaluated on 100 plants of each variety in the third year after planting in Northern Espírito Santo state

| Varieties | $\hat{m}$ | min | max | s | $CV_{(\%)}$ |
|---|---|---|---|---|---|
| **Stem diameter (cm)** | | | | | |
| Eva | 9.22 | 6.01 | 12.18 | 0.92 | 9.98 |
| Julieta | 7.35 | 5.52 | 9.64 | 0.80 | 10.86 |
| Princesa | 9.68 | 7.49 | 12.03 | 0.88 | 9.08 |
| **Fruits per plant (N°)** | | | | | |
| Eva | 285.83 | 71 | 459 | 70.25 | 24.58 |
| Julieta | 229.18 | 66 | 468 | 73.92 | 32.25 |
| Princesa | 160.04 | 68 | 272 | 46.00 | 28.75 |
| **Production (kg planta$^{-1}$)** | | | | | |
| Eva | 33.48 | 8.42 | 53.38 | 8.33 | 24.88 |
| Julieta | 31.87 | 8.97 | 63.62 | 10.28 | 32.25 |
| Princesa | 17.47 | 7.41 | 29.65 | 5.13 | 29.38 |

Another finding is that higher values of $\hat{\beta}_1$ contribute to increasing the optimal plot size (X0), which can be seen when analyzing the mass production per plant characteristic in the Julieta variety. In this case, analyzing the equations in Table 3, $\hat{\beta}_1 = 0.2790$ using the original method of Meier and Lessman[11] gives us $X_0 = 4.83$ and when using the bootstrap simulation method according to Celanti et al[13], $\hat{\beta}_1 = 0.4992$ and gives us an $X_0 = 5.91$, which corresponds to the increase of one plant per plot in the optimal size.

Regarding $X_0$, determined both by the maximum curvature method modified according to Meier and Lessman[11] and by the maximum curvature method modified with bootstrap simulation according to Celanti et al[13], it is clear that the $X_0$ was different between the characteristics, being higher for production characteristics and lower for stem diameter (Table 3). Therefore, if the objective was only to evaluate the diameter of the stem, in the three varieties, the installation of experiments with the evaluation of three plants per plot would be sufficient. However, in the case of evaluating stem diameter and productive characteristics, it would be necessary to set up an experiment with six plants per plot.

The demonstration of the relationship between plot sizes and CV values, together with the plot of the optimal plot size ($X_0$) is presented in Figure 2. It can be seen that the relationship is non-linear and that the use of plot size in addition will result in a larger plot size with a small gain in experimental precision, showing the importance of this type of work for further works with apple trees in the field.

The comparison between means using the Tukey test is shown in Table 4 for stem diameter. The use of six plants per plot, which is the optimal size, enabled us to see that the stem diameter is similar between the Eva and Princesa varieties and both have a greater stem diameter value than the Julieta variety. When comparing varieties using only one plant per plot, no change was observed in the comparison results. In a way, this behavior was expected given the fact that the optimal size for stem diameter was only three plants per plot. Whether the number of repetitions is increased from seven to 100, the analysis allows the

**Table 3:** Estimated equation, coefficient of determination ($R^2$), heterogeneity index (b), optimal plot size ($X_0$) and coefficient of variation for three apple tree varieties that are not very demanding in cold, in the third year after planting in Northern Espírito Santo state, using the original methodology of Meier and Lessman (ML) and the methodology of Meier and Lessman with bootstrap simulation (ML boot)

| Variedade | Methodologies[1] | Equations | $R^2_{(\%)}$ | b [2] | $X_0$ | $CV_{X0}$ |
|---|---|---|---|---|---|---|
| **Stem diameter (cm)** | | | | | | |
| Eva | ML | $\hat{Y} = 9.8205x^{-0.2920}$ | 0.8374 | 0.5841 | 1.96 | 8.02 |
| | $ML_{boot}$ | $\hat{Y} = 10.0024x^{-0.5041}$ | 0.9994 | 1.0082 | 2.72 | 6.04 |
| Julieta | ML | $\hat{Y} = 10.6676x^{-0.3944}$ | 0.9650 | 0.7887 | 2.52 | 6.92 |
| | $ML_{boot}$ | $\hat{Y} = 10.9147x^{-0.5071}$ | 0.9997 | 1.0142 | 2.89 | 6.37 |
| Princesa | ML | $\hat{Y} = 9.4069x^{-0.5336}$ | 0.9788 | 1.0672 | 2.68 | 5.23 |
| | $ML_{boot}$ | $\hat{Y} = 9.0427x^{-0.4940}$ | 0.9995 | 0.9880 | 2.52 | 5.72 |
| **Fruits per plant (N°)** | | | | | | |
| Eva | ML | $\hat{Y} = 23.8448x^{-0.3678}$ | 0.9719 | 0.7356 | 4.37 | 14.32 |
| | $ML_{boot}$ | $\hat{Y} = 24.3456x^{-0.4959}$ | 0.9991 | 0.9918 | 4.90 | 11.07 |
| Julieta | ML | $\hat{Y} = 32.0789x^{-0.2945}$ | 0.9875 | 0.5890 | 4.92 | 19.97 |
| | $ML_{boot}$ | $\hat{Y} = 31.3515x^{-0.4992}$ | 0.9986 | 0.9984 | 5.78 | 13.38 |
| Princesa | ML | $\hat{Y} = 28.4952x^{-0.4241}$ | 0.8919 | 0.8482 | 5.23 | 14.40 |
| | $ML_{boot}$ | $\hat{Y} = 28.5390x^{-0.4949}$ | 0.9992 | 0.9898 | 5.45 | 12.33 |
| **Production in the number (kg planta$^{-1}$)** | | | | | | |
| Eva | ML | $\hat{Y} = 25.1773x^{-0.5932}$ | 0.9913 | 1.1863 | 5.17 | 9.69 |
| | $ML_{boot}$ | $\hat{Y} = 24.2747x^{-0.4883}$ | 0.9983 | 0.9763 | 4.87 | 11.20 |
| Julieta | ML | $\hat{Y} = 32.5098x^{-0.2790}$ | 0.9290 | 0.5581 | 4.83 | 20.70 |
| | $ML_{boot}$ | $\hat{Y} = 32.1569x^{-0.4992}$ | 0.9999 | 0.9984 | 5.91 | 13.24 |
| Princesa | ML | $\hat{Y} = 29.3206x^{-0.4128}$ | 0.9506 | 0.8256 | 5.29 | 15.08 |
| | $ML_{boot}$ | $\hat{Y} = 28.7575x^{-0.4915}$ | 0.9997 | 0.9830 | 5.47 | 12.47 |

[1] Method: modified maximum curvature, according to Meier and Lessman[11]; $ML_{boot}$ = modified maximum curvature, according to Meier and Lessman[11] using bootstrap resampling simulation with 2000 simulations according to Celanti.[13]
[2] b: Smith heterogeneity index[18]

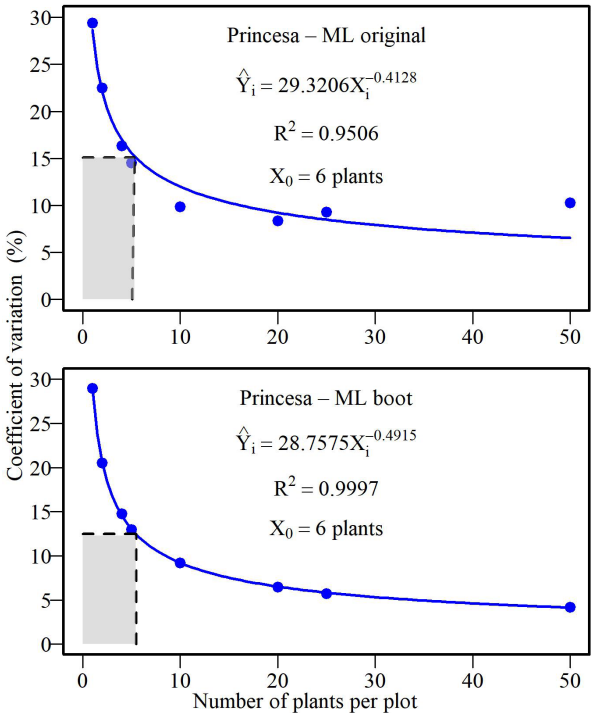


**Figure 4**: Graphical representation of the relationship between the coefficient of variation and plot size in the number of plants per plot, an equation that represents the relationship using the Meier and Lessman method ($\hat{Y}_i$), coefficient of determination ($R^2$), and the optimal plot size ($X_0$) for production per plant of the variety of Princesa apple tree, evaluated in the third year after planting in Northern Espírito Santo state [upper part of the original method of Meier and Lessman[11] and the bottom is the method with bootstrap simulation according to Celanti et al[13]].

**Table 4:** Comparison of means, using the test of Tukey, for stem diameter (cm) among three apple tree varieties that are not very demanding in cold, evaluated in the third year after planting, in northern Espírito Santo state, considering different numbers of repetitions and plot sizes

| Varieties | Characterization of the design [1] | | | |
|---|---|---|---|---|
| | Treatments = I | I = 3 | I = 3 | I = 3 |
| | Repetitions = J | J = 100 | J = 7 | J = 7 |
| | Plot size = $X_1$ | $X_1$=1 | $X_1$=1 | $X_1$=6 |
| Eva | | 9.22b | 9.22a | 9.22a |
| Julieta | | 7.33c | 7.33b | 7.33b |
| Princesa | | 9.67a | 9.67a | 9.67a |
| LSD [2] | | 0.29 | 1.19 | 0.67 |

[1] Means followed by the same letter are not different from each other by the test of Tukey at 5% probability.
[2] LSD = least significant difference by the test of Tukey, at 5% probability at the completely Random experimental design.

distinction between the three varieties, ranking Princesa as the one with the largest diameter.

It can be seen in Table 5 a comparison between the means of the three varieties for production in the number of fruits per plant. Using the optimal size of six plants per plot allowed us to observe a distinction among the three varieties, which was not possible to distinguish when the plot size was reduced to one plant, with the same seven replications. The increase to 100 repetitions of one plant per plot did not result in any change concerning the situation using the optimal size. This reinforces what was proposed by Meier and Lessman[11] of using the optimal plot size. Also, from another perspective of comparison, using the optimal plot size, in this example, 42 plants per variety would be used, which is less than half of the 100 plants used in the case of 100 repetitions of a plant.

Table 6 shows the comparison between the means of the varieties for mass production per plant. In the three

**Table 5:** Comparison of means, using the test of Tukey, for production (Fruits plant$^{-1}$) among three apple tree varieties that are not very demanding in cold, evaluated in the third year after planting, in northern Espirito Santo state, considering different numbers of repetitions and sizes of installment

| Varieties | Characterization of the design[1] | | | |
|---|---|---|---|---|
| | Treatments = I | I = 3 | I = 3 | I = 3 |
| | Repetitions = J | J = 100 | J = 7 | J = 7 |
| | Plot size = $X_1$ | $X_1$=1 | $X_1$=1 | $X_1$=6 |
| Eva | | 285.83a | 285.83a | 285.83a |
| Julieta | | 229.18b | 229.18ab | 229.18b |
| Princesa | | 160.04c | 160.04b | 160.04c |
| LSD [2] | | 21.38 | 87.56 | 47.50 |

[1] Means followed by the same letter are not different from each other by the test of Tukey at 5% probability.
[2] LSD = least significant difference by the test of Tukey, at 5% probability at the completely Random experimental design.

**Table 6:** Comparison of means, using the test of Tukey, for production (kg plant$^{-1}$) among three apple tree varieties that are not very cold demanding, evaluated in the third year after planting, in northern Espírito Santo state, considering different numbers of repetitions and plot sizes

| **Varieties** | **Characterization of the design [1]** | | | |
|---|---|---|---|---|
| | Treatments = I | I = 3 | I = 3 | I = 3 |
| | Repetitions = J | J = 100 | J = 7 | J = 7 |
| | Plot size = $X_1$ | $X_1$=1 | $X_1$=1 | $X_1$=6 |
| Eva | | 33.48a | 33.48a | 33.48a |
| Julieta | | 31.87a | 31.87a | 31.87a |
| Princesa | | 17.47b | 17.47b | 17.47b |
| LSD (2) | | 2.62 | 10.74 | 5.85 |

[1] Means followed by the same letter are not different from each other by the test of Tukey at 5% probability.
[2] LSD = least significant difference by the test of Tukey, at 5% probability at the completely Random experimental design.

simulation situations, the mean of the Eva and Julieta varieties are equivalent and both are superior to the Princesa variety. The means of the productivity of the three varieties exceeds that observed by other researchers[6,22] for plants of the same age, in other geographic regions of Brazil, showing a good adaptation to cultivation in the north from the state of Espírito Santo. The difference of almost 15 kg plant$^{-1}$ of the Princesa variety concerning Eva and Julieta may be due to some asynchrony in pollination since Princesa was later. It is reported that the Julieta variety was the earliest followed by Eva and Princesa was the latest. Lopes et al[6] report that the Princesa variety is more used as a pollinator and not as a producing variety and therefore it is expected that lower yields will be obtained with it.

Based on the outcome of this work, it is recommended to use Meier and Lessman' method[11] with bootstrap simulation, according to Celanti et al[13] reinforced by the fact that it is, concerning the original method, the least expensive, since it is not necessary to identify the position of the plants in the experimental area.

## CONCLUSIONS

The optimal size of plots with apple trees of the Eva, Julieta, and Princesa varieties that are not very demanding in the cold is six plants per experimental plot. The use of the maximum curvature method modified by Meier and Lessman[11] with bootstrap simulation according to Celanti et al[13] is recommended.

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