

Assessing equality of production and internal structure of twin plots in clonal eucalypt plantations: analyzing early measurements

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FOREST MANAGEMENT

ABSTRACT

Background: Extensive research seeks to improve forest management by understanding the effects of silvicultural treatments on productivity. Continuous forest inventory (CFI) plots offer valuable data for such studies. However, accuracy relies heavily on the twin plot concept, where two adjacent plots (twins) are established to isolate treatment effects from natural variation. This study explored the validity of the twin plot concept by analyzing data from 191 plot pairs in clonal eucalypt plantations. Specifically, it aimed to assess the equality of production volume and the internal structure between CFI plots and their twin plots.

Results: Normality assumptions for plot volume differences were not met, even after applying a transformation procedure. Paired t-tests couldn't be performed, but the non-parametric Wilcoxon test indicated no statistical difference in plot volumes. However, a different procedure called the L&O test revealed significant statistical differences. Gini coefficients demonstrated variations in tree volume distribution between plot pairs. Limited tree numbers and varying diameter classes prevented the use of Chi-square tests for diameter distribution equality. The Kolmogorov-Smirnov test showed non-adherence to estimated distributions using the Weibull distribution function. The L&O test identified significant differences in diameter distributions in 55 of the 191 plot pairs.

Conclusions: We have concluded that it is critical to determine the twin nature of plots during first tree measurement to properly analyze the effects of silvicultural treatments on forest productivity. This requires robust statistical tests, adherence to assumptions, examination of internal plot structures, and adequate plot sizes for modeling diameter distribution.

Keywords: continuous forest inventory; silvicultural treatments; forest productivity; forest management; statistical analysis.

HIGHLIGHTS

Assessing production and structure equality in CFI and twin plot pre-treatment.
Inadequacy of the t-test for paired data in the eucalypt plantation experiment.
Small plots do not allow for more robust statistical tests.
The internal structure is pivotal in evaluating CFI and twin plot equality.

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INTRODUCTION

In even-aged stands managed for commercial purposes, knowledge of the response to fertilization and weed control is important for making decisions about investments in silvicultural practices (Hakamada *et al.*, 2015).

One alternative for assessing the effect of fertilization and weed competition on productivity is the establishment of experimental plots called "twin plots" or "twin-plots" (Stape *et al.*, 2006). When paired with continuous forest inventory (CFI) plots, twin plots serve as control plots for the experiment.

In experiments of this nature, it has been common to use the paired t-test to verify the initial equality of CFI and twin plots in terms of wood productivity per unit area.

However, considering that productivity depends on the structural heterogeneity of the stand (Bourdier *et al.*, 2016; Forrester and Bauhus, 2016; Soares *et al.*, 2016; Soares, 2017), the diameter distribution needs to be considered in defining plot equality. Experimental plots with the same production per area can have different internal structures, that is, different diameter distributions, resulting in potential differential growth over time, given the different growth rates in different diameter classes.

In addition to considering the complete structure of the stands, there is also a need for the use of more rigorous statistical tests and procedures, in accordance with the hypotheses to be tested and the assumptions for their application. Thus, in studies involving twin plots, it is necessary to ensure, through careful analysis, the equality between the experimental plots (twins) paired with the continuous forest inventory (CFI) plots at the beginning of the assessments, prior to the application of silvicultural treatments.

Considering the above, this study aimed to evaluate the equality in terms of volume production and internal structure of the continuous forest inventory (CFI) plots and twin plots before the application of silvicultural treatments

at the beginning of the experiment on forest productivity, using different statistical tests and procedures.

The hypothesis of this study is that the equality of mean volumetric productions observed in two plots is sufficient to consider them as twin plots.

MATERIAL AND METHODS

Description of the study area

The clonal eucalypt stands used in this study are located in the southernmost region of the state of Bahia, Brazil. The five municipalities included in the study are Eunápolis, Guaratinga, Itabela, Itagimirim, and Santa Cruz Cabrália. The predominant soils in the region are argisols (mainly dystrophic yellow argisol and dystrophic red-yellow argisol) and latosols (Santos *et al.* 2018). The climate is classified as Af type, which is hot and humid with a small dry season. The average annual temperature is 25–27°C, and the average annual precipitation is 1256 mm. The majority of the precipitation occurs from November to April.

Data description

A total of 382 plots (191 CFI and 191 twin plots) were established in the study area. Each plot was 264 m² in size and consisted of two rows of 11 planting pits for a total of 22 planting pits per plot. The planting spacing was 5.0 x 2.4 m.

For each CFI plot, a corresponding twin plot of the same size and shape was established at a distance of 15 to 30 m from one side of the CFI plot (Stape *et al.* 2006). Silvicultural treatments such as fertilization and weed control were applied within the twin plots. Each selected CFI plot and its respective twin plot constitute a "Case *i*", where *i* = 1, 2, ..., 191 (Figure 1).

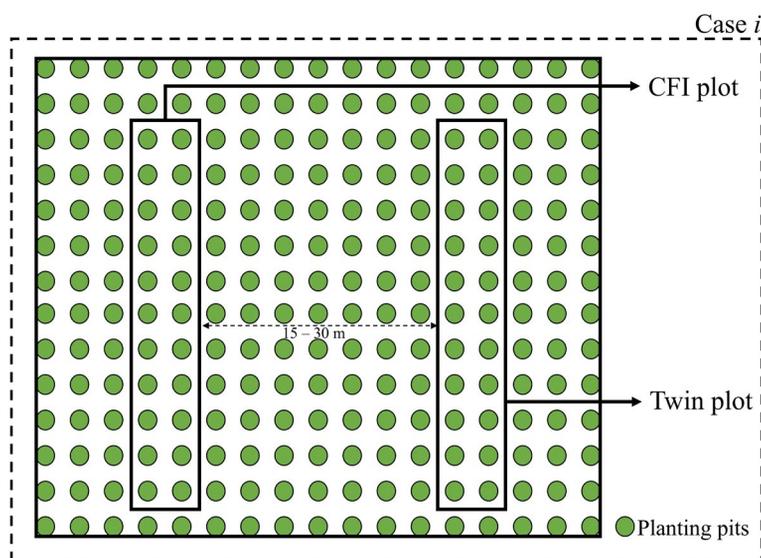


Figure 1: Scheme represented for CFI and twin plots for each Case *i*.

The diameter at breast height (dbh) of all trees, the total height of the first ten trees, and the total height of four dominant trees were measured in all 382 plots (CFI and twin plots). These measurements were taken at ages ranging from 1.64 to 12.22 years. However, only the data from the first measurement (< 24 months) were used for this study.

Prediction of total tree heights and volumes

The total heights of the trees not measured within the plots were predicted using a multilayer perceptron artificial neural network (ANN). The ANN was trained using data from 11,660 records of total heights collected throughout the inventoried area. The data were split into 70% for training and 30% for validating the configured neural networks. The continuous input variables for the network were tree age, *dbh*, maximum diameter, mean square diameter (*q*), and dominant height (*hd*) of the plot. The categorical variables included the cutting cycle, clone, and planting spacing.

The training algorithm for the ANN was Resilient Propagation RPROP+. The activation function was logistic, and the number of neurons in the hidden layer was 8. The stopping criterion for training was either the number of epochs equal to 3000 or the occurrence of a mean error equal to 0.0001, whichever came first (Casas et al., 2022, 2023).

For the prediction of individual tree volumes within the plots (bark volume for a minimum diameter of 6 cm), an ANN was also configured and trained. The input variables included age, *dbh*, total height (*ht*), and *dbh* /*ht* ratio (continuous variables), as well as rotation, cutting cycle, clone, and planting spacing (categorical variables). The training algorithm, activation functions, and stopping criterion were the same as those used for predicting total height.

The best artificial neural networks were selected during the validation process based on the correlation ($r_{y\hat{y}}$) between the observed (y) and estimated (\hat{y}) values, the root mean square error (*RMSE%*), and a graphical analysis of the relative percentage errors calculated by $RE\% = 100(\hat{y} - y)/y$.

Evaluation of Equality between Twin Plots during Installation

The equality between the two categories of plots (CFI and twin plots) was assessed in the first measurement, prior to the application of silvicultural treatments, considering the volume per unit area (m^3ha^{-1}) and the internal structure of the plots, through the analysis of volume and tree diameter distributions.

The assessment of equality between CFI and twin plots in terms of volume per hectare is typically performed using a paired t-test (Stape et al., 2006). In this case, the test is equivalent to the analysis of a randomized complete block design (RCBD), where each pair of plots constitutes a block with two treatments (CFI and twin plot). Thus, for one degree of freedom for treatment, the squared t-statistic value is equal to the F-statistic value from the analysis of variance (ANOVA) of the RCBD.

Therefore, before applying the t-test, the Lilliefors normality test was conducted on the differences between the volumes of CFI plots and twin plots. If the normality assumption was met, taking into account the data in the original unit (m^3ha^{-1}) or after transformation using the method suggested by Box and Cox (1964), the paired t-test would be used to evaluate the following null hypothesis (H_0): the mean difference between the volumes of the two types of plots is equal to zero. If the normality assumption was not met, the nonparametric Wilcoxon test would be applied (Wilcoxon, 1945).

Alternatively, the procedure proposed by Leite and Tavares de Oliveira (2002) (L&O) could be used, which combines the modified Graybill's F-test (F_{H_0}), the t-test for mean error (t_e), and the coefficient of linear correlation between the observed volumes in the CFI plots and twin plots ($r_{y\hat{y}}$), to also assess the equality of plot volumes.

The eight scenarios involving the three test statistics that are detailed in Table 1 provided the basis for the L&O procedure's decision rule. Only situation 1 indicates equality between the methods. In this study, the hypothesis of equality between the volumes in each pair of CFI plots and twin plots or *Cases*.

In terms of internal structure, the uniformity of the distribution of individual tree volumes in each pair of plots (CFI and twin) was assessed using the Gini coefficient (Farris, 2010).

Table 1: Rule of decision for comparison of analytical methods (Leite and Tavares de Oliveira, 2002).

Situation	$F(H_0)$	t_e	$r_{y\hat{y}}$	Decision
1	ns	Ns	$r_{y\hat{y}} > 1 \bar{\epsilon} $	$Y_j = Y_i$
2	ns	Ns	$r_{y\hat{y}} \leq 1 \bar{\epsilon} $	$Y_j \neq Y_i$
3	ns	*	$r_{y\hat{y}} > 1 \bar{\epsilon} $	$Y_j \neq Y_i$
4	ns	*	$r_{y\hat{y}} \leq 1 \bar{\epsilon} $	$Y_j \neq Y_i$
5	*	Ns	$r_{y\hat{y}} > 1 \bar{\epsilon} $	$Y_j \neq Y_i$
6	*	Ns	$r_{y\hat{y}} \leq 1 \bar{\epsilon} $	$Y_j \neq Y_i$
7	*	*	$r_{y\hat{y}} > 1 \bar{\epsilon} $	$Y_j \neq Y_i$
8	*	*	$r_{y\hat{y}} \leq 1 \bar{\epsilon} $	$Y_j \neq Y_i$

ns and * denote, respectively, not significant and significant at a level α of probability.

The Gini coefficient is a statistical tool for measuring income or wealth inequality in a population. Created by Italian statistician Corrado Gini in 1912, it ranges from 0 to 1, with 0 signifying perfect equality (everyone earns the same) and 1 indicating complete inequality (one individual holds all the income).

The Gini Coefficient was calculated to compare the inequality between pairs of plots (IFC and twin) during the first measurement. For its calculation, the first step involved organizing the trees in each plot in descending order based on the value of the variable of interest (in this case, the bark volume of each individual tree). Thus, the plot served as the unit for which the inequality was calculated.

Next, the cumulative value of the number of trees and the cumulative value of the individual tree volumes in the plot were calculated, allowing for the relative cumulative values for the number of trees and the volume of the trees to be obtained.

The area of inequality was determined by subtracting the area under the Lorenz Curve (A) from the total area below the Perfect Equality Line. The calculation of the area was approximately obtained using the trapezoidal method: the sum of the areas of the n trapezoids under the Lorenz Curve.

$$A = \sum_{i=1}^n (Y_{i+1} - Y_i) \left(\frac{X_i + X_{i+1}}{2} \right) \quad (1)$$

Where $(Y_{i+1} - Y_i)$ is the height of trapezoid i ; $(X_i - X_{i+1})$ is the longer and shorter bases of trapezoid i , and n is the number of trapezoids, respectively.

The two-parameter Weibull function was fitted to the data from the first measurement of each experimental plot to characterize the diameter distribution prior to treatment application. The goodness of fit was assessed using the Kolmogorov-Smirnov (K-S) test. This same test was also used to evaluate the hypothesis H_0 : The estimated distribution of IFC and twin plots, in each case, does not differ statistically at a 5% significance level; H_a : H_0 is not true. Additionally, the Chi-Square test and the statistical procedure proposed by Leite and Tavares de Oliveira (2002) were applied to assess the hypothesis H_0 : The observed diameter distribution in the IFC plot does not differ statistically from the observed distribution in the twin plot,

in each case; H_a : H_0 is not true. A significance level of 5% was used in all statistical analyses.

RESULTS

Artificial Neural Networks

The trained and validated ANNs for estimating the total heights and volumes of trees in the continuous forest inventory (CFI) and twin plots are described in Table 2.

The graphs (Figures 2a and 2b) show that, for the variable of total height, 90.5% of the trees had relative errors in the estimates within $\pm 5\%$. For volume (Figures 2c and 2d), the relative errors ranged between $\pm 5\%$ for 65.5% of the trees. These results, combined with the magnitude of the errors, indicate satisfactory accuracy in the estimates of height and volume of the trees and, as a consequence, in the estimates of production per unit area ($\text{m}^3 \text{ha}^{-1}$).

Evaluation of production per unit area

Figure 3 shows considerable variability in the volumes of the plots, ranging from approximately 100 to 800 m^3/ha , likely due to the forest management applied. The volumes per hectare of the continuous forest inventory (CFI) and twin plots were concentrated around a 45-degree line, indicating the possibility of equality in the estimates. We have also seen that the variability becomes more pronounced at higher volumes, especially above 600 m^3/ha , highlighting the importance of conducting proper statistical tests.

Despite a strong correlation between the two types of plots, the Lilliefors test rejected the hypothesis that the differences between the volumes of the CFI and twin plots could be studied with a normal distribution (p -value < 0.01). This hypothesis was also rejected for the data transformed according to Box and Cox (1964).

Based on the results of the normality test of the data, the Wilcoxon test (Wilcoxon 1945) was applied, resulting in a p -value of 0.7379. Therefore, according to this test, the volumes of the twin plots in the first measurement of the experiment were considered statistically equal to the corresponding volumes of the CFI plots.

Table 2: Description of the selected artificial neural networks for estimating the height and volume of individual trees and their respective accuracy measures in the validation data.

Characteristic	Description	
	ANN Height	ANN Volume
Architecture	<i>Multilayer perceptron – MLP</i>	<i>Multilayer perceptron - MLP</i>
Number of neurons in the input, intermediate, and output layers, respectively	32-8-1	56-25-1
Continuous Variable Inputs	Age, <i>dbh</i> , <i>dbhmax</i> , q , h_d	Age, <i>dbh</i> , h_t , relationship <i>dbh/h_t</i>
Categorical Variable Inputs	Cutting cycle, Clone, Spacing	Rotation, Cutting cycle, Clone, Spacing
Root Mean Square Error (RMSE)	0.875	0.025
Correlation coefficient (r_{yy})	0.9904	0.9948

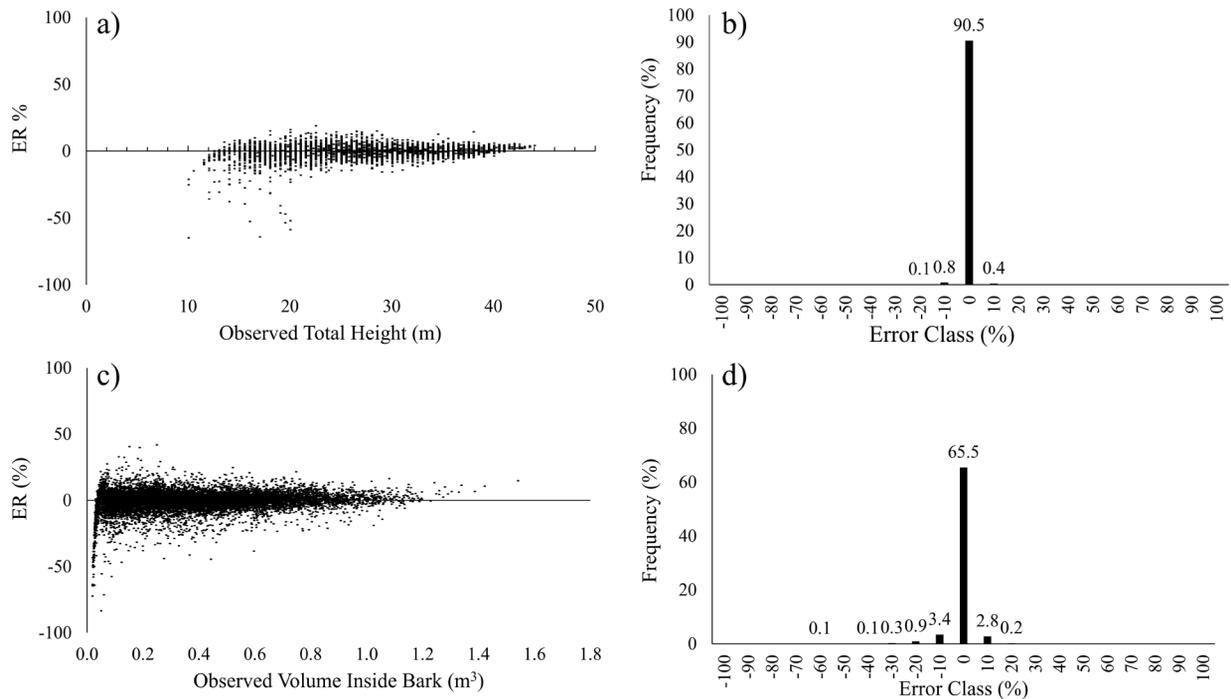


Figure 2: Error distributions as relative percentages in relation to the observed total height (a) with its error frequency histogram (b), and in relation to the observed volume inside bark (c) with its error frequency histogram (d) in the validation data.

However, the L&O procedure showed a statistical difference between the volumes of the two plot types (situations 2 to 8 in the decision rule). This is due to the fact that, as the trend line in Figure 3 illustrates, the volumes of the twin plots are greater in plots with lower volumetric stock and higher in sites with larger stock.

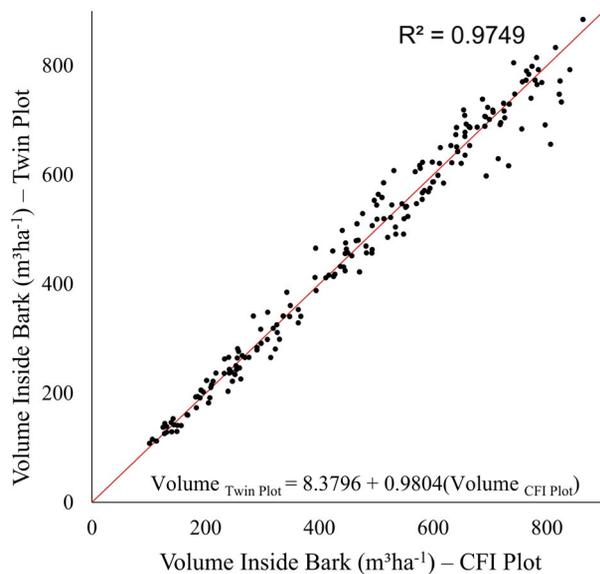


Figure 3: Scatter plot of volumes of twin plots in relation to the corresponding volumes of inventory plots (CFI) before the application of silvicultural treatments.

Evaluation of the internal structure of the plots

The wide variation of Gini coefficients among the cases (CFI and twin plots) (Figure 4) shows that there is heterogeneity in the conditions of the locations where they were located. Additionally, differences in coefficients were also seen within the same pair of plots or cases, indicating the non-uniformity of the initial conditions (before the application of treatments) of the CFI and twin plots in terms of tree volumes.

In percentage terms, the difference between the Gini coefficients within the same pair of plots or Case ranged from -65% to 325%, indicating a wide range of volume heterogeneity (Figure 5). This result indicates that at the beginning of the experimental setup, some CFI plots had a more uniform distribution of tree volumes compared to the twin plots, and vice versa.

Regarding the diameter distribution, there was no adherence of the estimated distributions using the Weibull distribution function in the pairs of plots (CFI and twins), considering the Kolmogorov-Smirnov test (p-value < 0.05). The small number of individuals (n = 22) and the different numbers of diameter classes prevented the evaluation of the equality of distributions using the Chi-Square test. This result allows inferring the inequality of diameter distributions in the plots (CFI and twins) at the time of experiment establishment (first plot measurement). Furthermore, it suggests that in other similar studies, the plot size should be sufficient to contain a larger number of trees, enabling the assessment of the internal structure of the two plot types (CFI and twins).

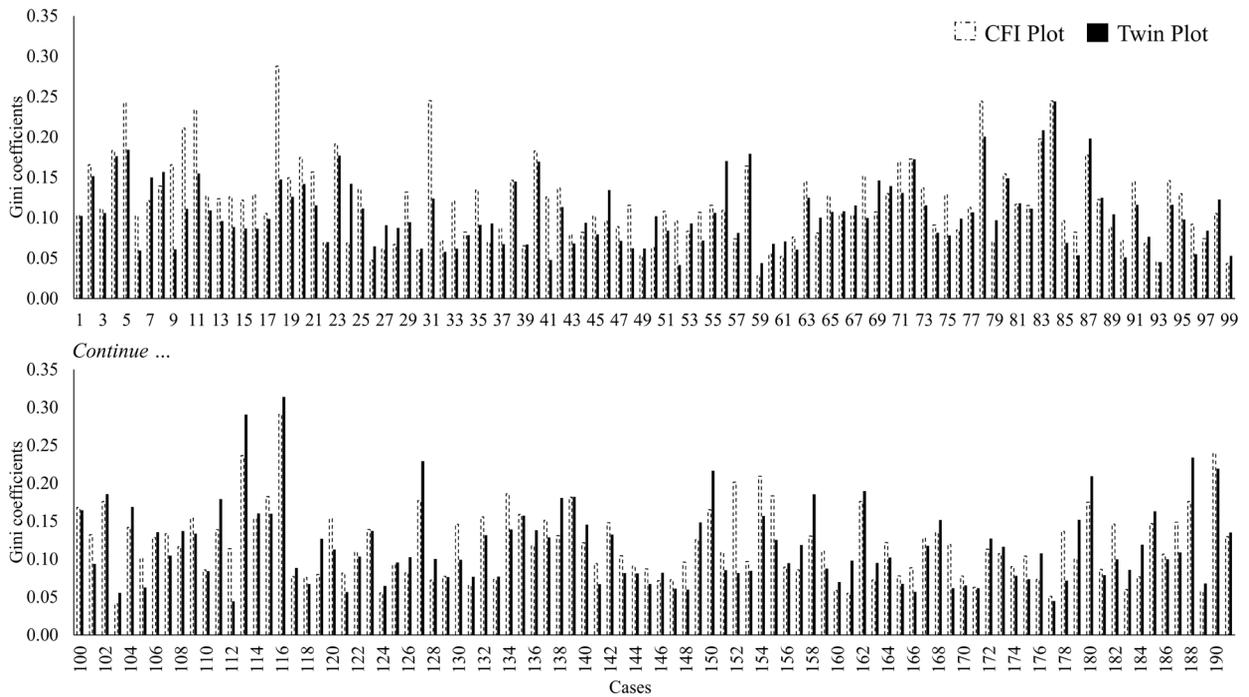


Figure 4: Gini coefficients calculated for the CFI and twin plots before the application of silvicultural for all cases.

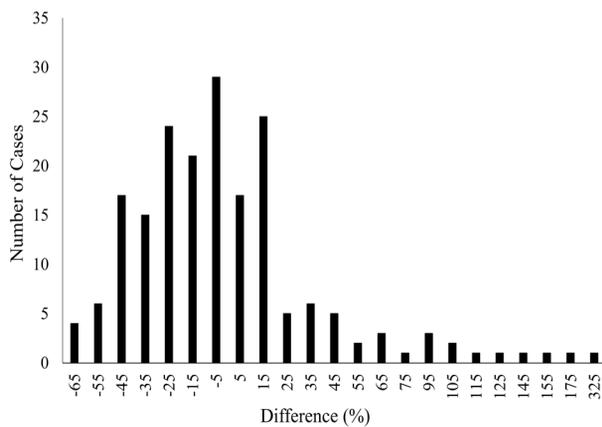


Figure 5: Distribution of percentage differences between the Gini coefficients of the CFI and twin plots in the 191 pairs of plots (cases).

As an example, consider the diameter distributions presented in Figure 6, where it can be observed that the mean diameters are the same in both plot types, while the maximum and minimum diameter values, kurtosis, and skewness differ between them. These discrepancies reinforce the conclusion that the plots have different structures before the application of treatments, and thus there is uncertainty in inferring treatment effects on the final age of evaluation.

By grouping the diameter data into classes with a width of 1 cm and applying the statistical L&O procedure, differences were observed between the distributions in 29% of the CFI and twin plot pairs (situations 2 to 8).

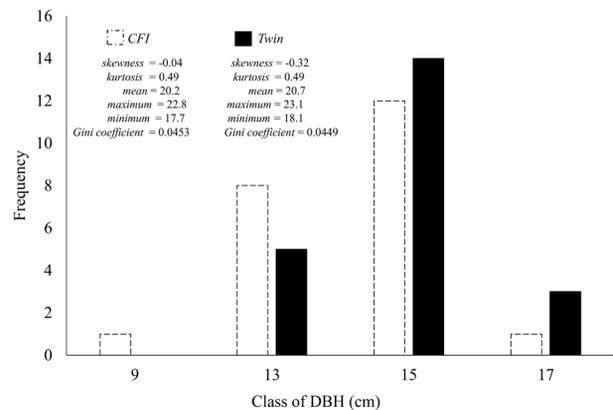


Figure 6: Observed frequencies of diameters and descriptive statistics for a pair of plots (CFI and twin).

Figure 7 graphically illustrates the methodology used to evaluate the homogeneity of production and internal structure in twin plots of clonal eucalyptus plantations.

DISCUSSION

Ensuring equality between CFI and twin plots is necessary to ensure future interpretations of treatment effects. Failing to conduct a robust verification can lead to uncertainty about the true treatment effects, which may be masked by potential differences, such as differences in the internal structures of the two plot types.

This study revealed limitations with traditional statistical tests, such as the paired t-test, which assumes

normality of the differences between plot volumes. This assumption is often unmet in ecological studies where natural variation and small sample sizes can distort data distribution, as highlighted by the results of the Lilliefors test and the failure of the Box-Cox transformation. This raises a broader issue about the adequacy of conventional statistical methods in ecological research.

In studies on forest productivity, it is common to apply the paired Student's t-test to evaluate the null hypothesis (H_0) that the mean differences in volumes ($m^3 ha^{-1}$), diameters, or heights between twin plots and inventory plots are statistically equal to zero at a given significance level (Stape *et al.*, 2006). However, it is observed in studies of this nature that this test is often applied without verifying its assumptions, such as the normality of the differences between the measured characteristics in the two plot types. Therefore, not meeting this assumption may lead to an incorrect application of the t-test for paired data, and it becomes necessary to transform the data or, alternatively, apply a nonparametric statistical test such as the Wilcoxon test (Wilcoxon, 1945), considering the possible loss of sensitivity of the t-test in the absence of normality (Montgomery, 2020).

It is worth noting that the result obtained in the normality test may have been influenced by the fact that this is not an experimental design in the strict sense, but rather a sampling design where small "experimental" plots were used with a small number of trees to characterize the initial equality of the plots.

According to Leite and Tavares de Oliveira (2002), inferring the equality of two sets of paired data based on hypotheses about the means or mean differences is not an efficient procedure. As an example, when applying the procedure proposed by these authors to compare volumes per hectare in twin and CFI plots, the hypothesis of equality between the two plot types was rejected, unlike the Wilcoxon test.

While the non-parametric Wilcoxon test was used to address the lack of normality, it still failed to detect significant differences in plot volumes that were uncovered by the

more robust L&O procedure. This discrepancy suggests that common statistical approaches, even after adjustments, may lack the sensitivity needed to detect differences in plot characteristics. The L&O procedure, which incorporates multiple statistical measures—such as Graybill's F-test, mean error t-test, and the correlation coefficient—provides a more nuanced approach that should be considered in future research. This raises an important point for forest science methodologies: relying solely on paired t-tests or simple non-parametric alternatives can obscure significant structural differences that may influence the outcomes of silvicultural experiments.

Regarding the diameter distributions of the plot pairs (CFI and twin), even with the limitation of the small plot size, the L&O procedure was effective in detecting differences between the distributions in the two plot types.

In forestry, the use of the Gini coefficient for studies of dominance and uniformity in plantations goes back a long time. This approach has been used for forest structure classification, carbon uptake flow classification (Safi and Mobitz, 2016), the assessment of competition among plants, and the influence of heterogeneity on the growth and productivity of eucalyptus plantations (Soares *et al.*, 2016).

The observation of high variability in the Gini coefficients between and within twin plots reveals significant differences in the structural uniformity of the experimental sites. This heterogeneity, indicated by the variations in diameter distributions and volume inequalities, suggests that the initial state of forest stands can significantly impact the results of silvicultural treatments. As Soares *et al.* (2016) indicated, structural heterogeneity in plantations directly affects growth dynamics and productivity, and this study corroborates those findings by showing that even before treatments, differences in tree size and distribution can create biases in productivity assessments.

The use of the Gini coefficient for measuring internal inequalities within the plots is a novel application within this context and underscores the importance of considering not only mean productivity but also the distribution of tree volumes. This metric, typically used in socio-economic

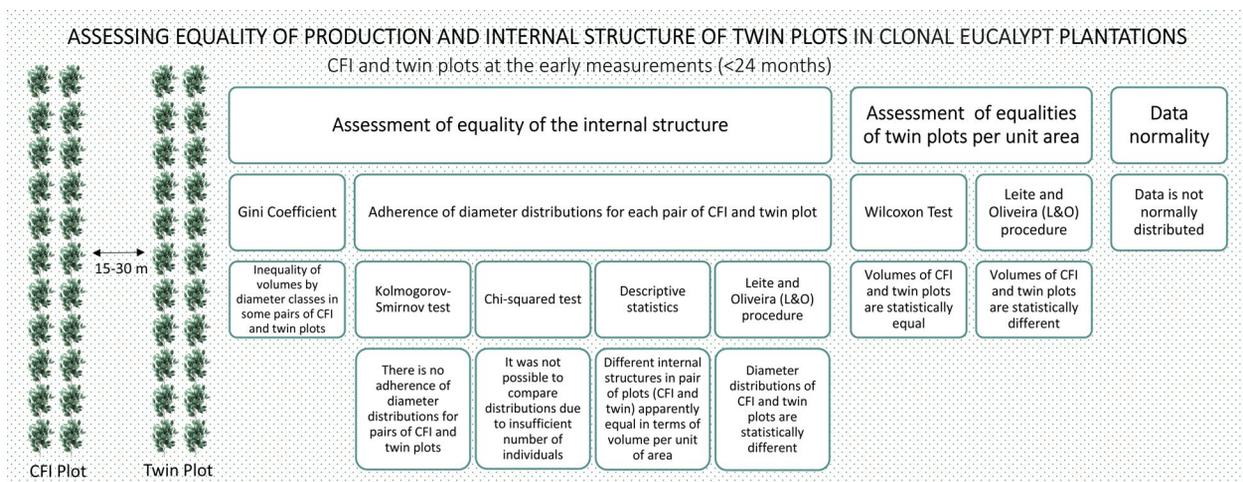


Figure 7: Graphical abstract of the research methodology and findings.

studies to measure income inequality, has shown its value in ecological research by offering insights into the competitive dynamics within stands. A high Gini coefficient, reflecting greater inequality, can point to uneven growth conditions that may lead to differential responses to silvicultural treatments. This insight supports the need for more rigorous controls in forest experiment design, where twin plots should be matched not just on overall volume but also on internal structure to avoid misinterpretation of treatment effects.

The results also show that in studies involving twin plots, the plot size must be sufficient to adequately characterize the internal structure of the plantations in terms of diameters. The plots should have a sufficient number of trees so that their diameter distribution can be described by a probability density function and allow for the application of statistical tests such as the chi-square test. In this study, in addition to a few individuals in some diameter classes, there were cases where certain diameter classes had no individuals, and there were also unequal diameter class intervals between plots.

The innovation of this study is its in-depth examination of both volume production and internal structure equality (e.g., diameter distribution) in twin plots. The study demonstrates that ensuring plot equality is not only about volume but also about understanding structural heterogeneity, an approach that is less common in similar research.

The implications of these findings are far-reaching for forest management and silvicultural practices. The study suggests that failure to control for internal plot structures can lead to misleading conclusions about the efficacy of treatments such as fertilization. For example, in cases where twin plots differ in their diameter distributions, responses to silvicultural interventions may vary due to inherent growth patterns rather than the treatment itself. This could result in suboptimal management decisions, where treatments are either prematurely discarded or overly promoted based on biased data.

Given the findings, forest managers should adopt a more comprehensive approach to plot selection and experimental design, ensuring that twin plots are not only volume-equivalent but also structurally comparable. This could be achieved by expanding plot sizes to include more trees, which would provide a more accurate reflection of the stand's structural diversity and allow for more robust statistical testing, such as the Chi-Square test for diameter distribution equality.

Several key areas must be explored more critically, such as the implications of plot heterogeneity, the limitations of current statistical methods, and the broader significance of the findings for forest management practices. Additionally, further investigation into the role of structural variation within twin plots can provide new insights into the management of clonal plantations.

Future studies should focus on creating more sophisticated statistical models that can handle the complexity and heterogeneity of forest data. This could include a combination of Bayesian methods, machine learning, and non-parametric tests to better capture variability in plot structures.

CONCLUSIONS

The hypothesis that the equality of mean volumetric productions observed in two plots is sufficient to consider them as twin plots was rejected.

The L&O statistical procedure combined with the Gini coefficient is more efficient than conventional statistical tests in identifying structural differences between continuous forest inventory plots and supposedly twin plots.

AUTHORSHIP CONTRIBUTION

Project Idea: CRR; CPBS; HGL.

Funding: CRR; CPBS; HGL.

Database: CRR; CPBS; HGL.

Processing: CRR; CPBS; HGL.

Analysis: CRR; CPBS; GFSS; HML; GGC; HGL.

Writing: CRR; GGC.

Review: CPBS; HGL.

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