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Original article

Comparison of non-destructive methods for estimating the leaf area of Cinchona officinalis L. using digital image processing

Comparación de métodos no destructivos para estimar el área foliar de Cinchona officinalis L. mediante procesamiento digital de imágenes

Comparação de métodos não destrutivos para estimar a área foliar de Cinchona officinalis L. por meio de processamento digital de imagens

Franklin Hitler Fernandez-Zarate[1], Annick Estefany Huaccha-Castillo[2], Segundo Primitivo Vaca-Marquina[3], Alejandro Seminario-Cunya[4], Víctor Hugo Taboada-Mitma[1], Luis Jhoseph Pérez-Delgado[1], David Coronel-Bustamante[1], Lenin Quiñones-Huatangari[5]*

[1]Yanayacu Experimental Center, Directorate of Supervision and Monitoring in Agricultural Experimental Stations, National Institute of Agricultural Innovation (INIA), Jaén San Ignacio Highway Km 23.7, Jaén 06801, Cajamarca, Peru.

[2]International Institute for Research on the Quina Tree, National University of Jaén, Cajamarca, Peru.

[3]National University of Cajamarca, Cajamarca, Peru.

[4]National Autonomous University of Chota, Cajamarca, Peru.

[5]Professional School of Data Science and Artificial Intelligence Engineering. Toribio Rodríguez de Mendoza National University of Amazonas, Peru.

*Corresponding author: fran.9615fernandez@gmail.com

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ABSTRACT

Cinchona officinalis It is an important plant species and was the only treatment for malaria for over three centuries. The aim of this study was to compare the accuracy of four non-destructive digital image processing methods (LeafArea and three ImageJ algorithms) for estimating the leaf area of young *C. officinalis* plantations under two establishment conditions: forest stands and enrichment strips. Leaves were photographed at a distance of 8 cm using a 24 MP smartphone and processed with the evaluated methods. Statistical analysis included box and whisker plots, Pearson correlation, and Friedman test. The results showed that ImageJ methods M3 and M4 had the highest accuracy (r = 0.99), with no significant differences between them, and overestimations detected in M1 and M2. It is concluded that M3 and M4 are fast, low-cost, and highly accurate options for foliar monitoring of *C. officinalis* in the field.

Keywords: Leaf area, Plant Resource, Technology, Quinine Tree, ImageJ.

RESUMEN

Cinchona officinalis es una importante especie vegetal, fue el único tratamiento para la malaria durante más de tres siglos. El objetivo de este estudio fue comparar la precisión de cuatro métodos no destructivos de procesamiento digital de imágenes (LeafArea y tres algoritmos de ImageJ) para estimar el área foliar de plantaciones jóvenes de *C. officinalis* en dos condiciones de establecimiento: macizo forestal y franjas de enriquecimiento. Se fotografiaron hojas a 8 cm de distancia utilizando un smartphone de 24 MP y se procesaron con los métodos evaluados. El análisis estadístico incluyó diagramas de caja y bigotes, correlación de Pearson y prueba de Friedman. Los resultados mostraron que los métodos M3 y M4 de ImageJ presentaron la mayor precisión (r = 0,99), sin diferencias significativas entre ellos, y con sobreestimaciones detectadas en M1 y M2. Se concluye que M3 y M4 son opciones rápidas, de bajo costo y alta precisión para el monitoreo foliar de *C. officinalis* en campo.

Palabras clave: Área foliar, Recurso Vegetal, Tecnología, Árbol de la Quina, ImagJ.

RESUMO

Cinchona officinalis é uma espécie vegetal importante, foi o único tratamento para a malária durante mais de três séculos. O objetivo deste estudo foi comparar a precisão de quatro métodos não destrutivos de processamento digital de imagens (LeafArea e três algoritmos do ImageJ) para estimar a área foliar de plantações jovens de *C. officinalis* em duas condições de estabelecimento: maciço florestal e faixas de enriquecimento. As folhas foram fotografadas a 8 cm de distância usando um smartphone de 24 MP e processadas com os métodos avaliados. A análise estatística incluiu diagramas de caixa e bigodes, correlação de Pearson e teste de Friedman. Os resultados mostraram que os métodos M3 e M4 do ImageJ apresentaram a maior precisão (r = 0,99), sem diferenças significativas entre eles, e com superestimativas detectadas em M1 e M2. Conclui-se que M3 e M4 são opções rápidas, de baixo custo e alta precisão para o monitoramento foliar de *C. officinalis* em campo.

Palavras-chave: Área foliar, Recurso Vegetal, Tecnologia, Árvore de Cinchona, ImagJ.

INTRODUCTION

Cinchona officinalis is a medicinal plant species known as "cascarilla," "quina," or "quinine tree" that belongs to the Rubiaceae family (Raheem *et al*., 2004). It is found naturally in the high Andean and Amazonian regions of Bolivia, Ecuador, Colombia, and Peru (Aymard 2019). Its importance lies in its bark, which contains several alkaloids, including quinine, which was used for more than three centuries as the sole treatment for malaria. This property led to the overexploitation of cinchona forests, bringing it to the brink of extinction (Kaufman and Rúveda 2005).

Leaf area (LA) is an important parameter for the analysis of interactions between the atmosphere and plants, explaining the physiological and agronomic behavior of plant species with respect to photosynthetic efficiency, evapotranspiration, amount of water, radiation and response to fertilization (Blanco and Folegatti 2005; Syvertsen, Goñi and Otero 2003).

There are two categories for measuring leaf area (LA): direct and indirect (Figure 1) (Arslan, Erten, and Inan 2021; Bréda 2003; Jonckheere *et al.,* 2004). Direct LA estimation can be destructive or non-destructive. Destructive methods require leaf removal or leaf-popping traps, are very expensive, and labor-intensive (Casa, Upreti, and Pelosi 2019; Garrigues *et al.,* 2008); these include methods based on leaf silhouette, applications, planimeters, and scanners. Indirect or non-destructive methods allow for repeated leaf measurements over time, avoiding the biological disturbance characteristic of destructive methods (Suárez *et al.,* 2018; Swart *et al.,* 2004), and are useful when there is a low density of plants under study (Blanco and Folegatti 2005) . These include the use of mathematical models, digital cameras, and apps.

There are electronic instruments that estimate AF (LI-3000C, CI-202, CI-203, LAI-2000, SunScan from Delta-T Devices) and prove to be an alternative to destructive methods (Campos-Taberner *et al.,* 2016; Jonckheere *et al.,* 2004; Weiss *et al.,* 2004); however, they tend to be expensive, their portability will depend on the work area, and maintenance usually requires a lot of time (Confalonieri *et al.,* 2013; Thimonier, Baker (1996), Castillo, *et al.* (2014) Liu *et al* (2010), Sedivy and Schleppi 2010; Yilmaz *et al.,* 2008).

In this context, the use of digital photography emerges as an option (Chianucci *et al.,* 2015) which are then processed in specialized image processing software, for example, ImageJ developed by the National Institutes of Health of the United States and which is in the public domain; it is also one of the most used in AF estimation (Easlon and Bloom 2014; Ferreira *et al.,* 2017; Rincón, Olarte and Pérez 2012) ImageJ uses a threshold-based pixel count index to determine AF (Easlon and Bloom 2014).

Furthermore, smartphone software packages (apps) have been developed (Confalonieri *et al.,* 2013; Qu *et al.,* 2021) to determine leaf area (LA) in various ecological studies (Teacher *et al.,* 2013). For example, LeafArea, launched in 2020 and created by Skyberry

, is a digital image analysis software that uses a series of thresholds and color relationships to estimate the LA of an image in a few seconds with minimal user intervention (Ahmad *et al.,* 2015; Easlon and Bloom, 2014). These tools offer an acceptable degree of accuracy, are easy to use, and inexpensive (Confalonieri *et al.,* 2013) . In this context, the objective of this research was to compare the accuracy of four non-destructive digital image processing methods (LeafArea and three ImageJ algorithms) for estimating the leaf area of young *C. officinalis plantations.* This study constitutes the first documented comparison of non-destructive methods for estimating leaf area in *C. officinalis* , providing practical criteria for their use in the field and contributing to improving the monitoring and management strategies of this endangered species (Figure 1).

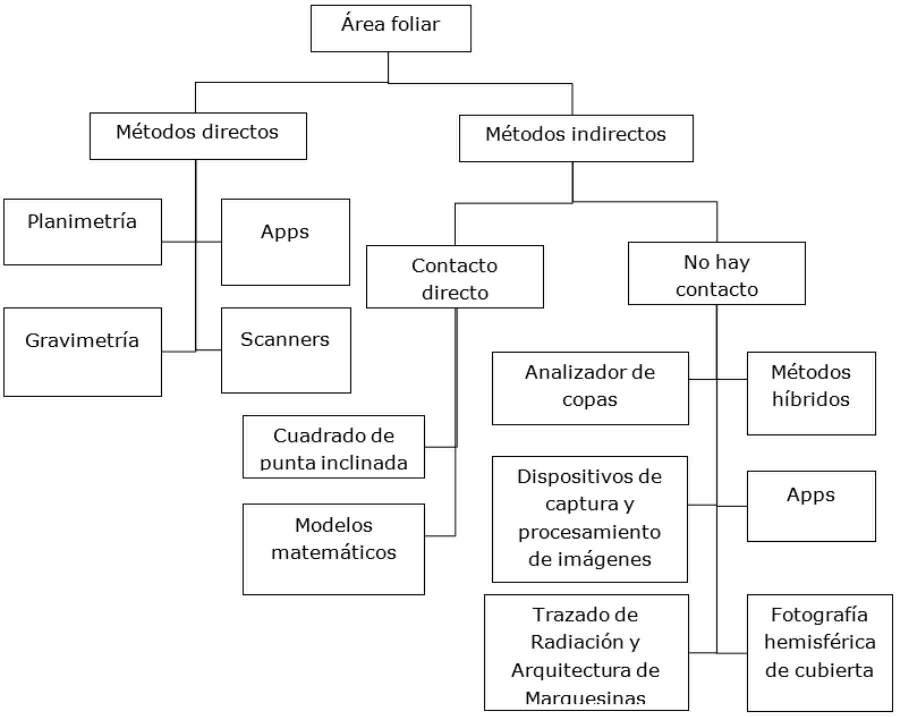

Figure 1. *- Methods for determining leaf area*

Source: (Jonckheere et al., 2004).

MATERIALS AND METHODS

Experimental site

Data was collected in January 2022 in a three-month-old *C. officinalis plantation located in the community of La Cascarilla (5°39'40.99''S and 78°54'35.14'W), Jaén Province, Peru. The area has an average altitude of 2,058 m and corresponds to a premontane humid forest* (bh -P). The maximum temperature is 20.5 °C and the minimum is 13 °C; annual rainfall is 1,730 mm (Fernández and Huaccha, 2022). The *C. officinalis* plantation was established as an enrichment strip (ES) and as a forest stand (FS).

Photo montage

A total of 96 randomly selected leaves (2 leaves per plant) were measured, covering different sizes and positions on the plant to ensure representativeness. Photographs were taken under diffuse natural light conditions, avoiding harsh shadows and reflections, and maintaining a fixed distance of 10 cm as the optimal standard for focus and scale calibration. The leaves were photographed against a white background (20 x 12 cm card) with a 2 cm long reference line drawn next to the leaf location to maintain scale during image processing. To flatten the leaves, they were covered with a 20 x 12 cm, 3 mm thick, frosted glass panel to prevent glare.

Leaf area measurement

A Huawei mobile phone with a 24-megapixel MAR-LX3A camera model was used to estimate the leaf area, which was measured in two ways:

LeafArea (M1) smartphone application was installed, the AF was directly determined by placing the equipment 10 cm from the plant leaf on the support to photograph it and finally the area estimated by the application was recorded.

Indirectly: Photographs were taken 10 cm from the plant leaf on the photographic support. These images were processed in the ImageJ software using three methods for determining the leaf area, following the procedures in the software: M2) File - Open - Image - Crop - Analyze - Set scale - Image - Type - 8-Bit - Image - Fit - Threshold - Analyze - Measure, M3) File - Open - Line width - Analyze - Set scale - Wand tool - Analyze -

Measure, M4) File - Open - Line width - Analyze - Set scale - Polygon selections - Analyze - Measure.

Statistical analysis

Box and whisker plots were used on the data matrix to visually describe characteristics such as the mean, quartiles, dispersion, and symmetry of the leaf area obtained by the four methods. To estimate the fit between each leaf area determination method, Pearson correlation coefficients were calculated (the normality of the variables was verified using the Kolmogorov-Smirnov test) between the area values obtained with each method. Finally, the non-parametric Friedman test was used to compare the medians of the four groups. THE ANALYSES WERE PERFORMED USING THE RStudio software.

RESULTS

Variability of leaf area according to method and type of planting

C. officinalis plants sown as a forest stand was 31.18 cm^2, recorded with M1, followed by the AF recorded with M2 (26.79 cm^2), while the minimum AF was recorded with M4 (3.37 cm^2) followed by M3 (3.45 cm^2). In *C. officinalis* plants sown in enrichment strips, the maximum AF was 83.29 cm^2, recorded with M2, followed by the AF recorded with M1 (60.23 cm^2), while the minimum AF was recorded with M4 (6.57 cm^2) followed by M3 (6.79 cm^2) (Figure 2A, B).

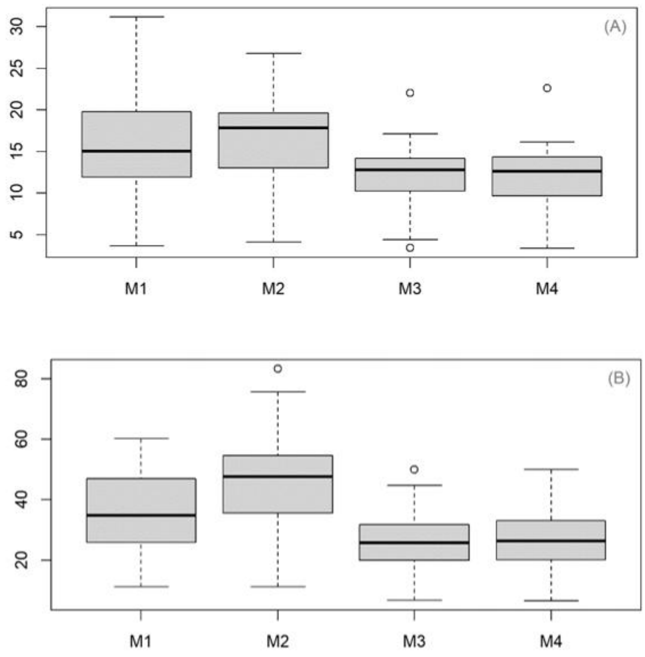

Figure 2. *- Box and whisker diagrams of the leaf area estimated by non-destructive methods in C. officinalis plants planted in forest stands (A) and in enrichment strips (B)*

Correlations between methods

C. officinalis plants sown as a forest stand, correlations close to one were calculated for methods M3 and M4 (r=0.99) and between M1 and M2 (r=0.85) (Figure 3A). While for the estimated AF in *C. officinalis plants* sown using enrichment strips, the two positive correlations close to one were observed between methods M3 and M4 (r=0.99) and between M2 and M4 (r=0.97) (Figure 3B).

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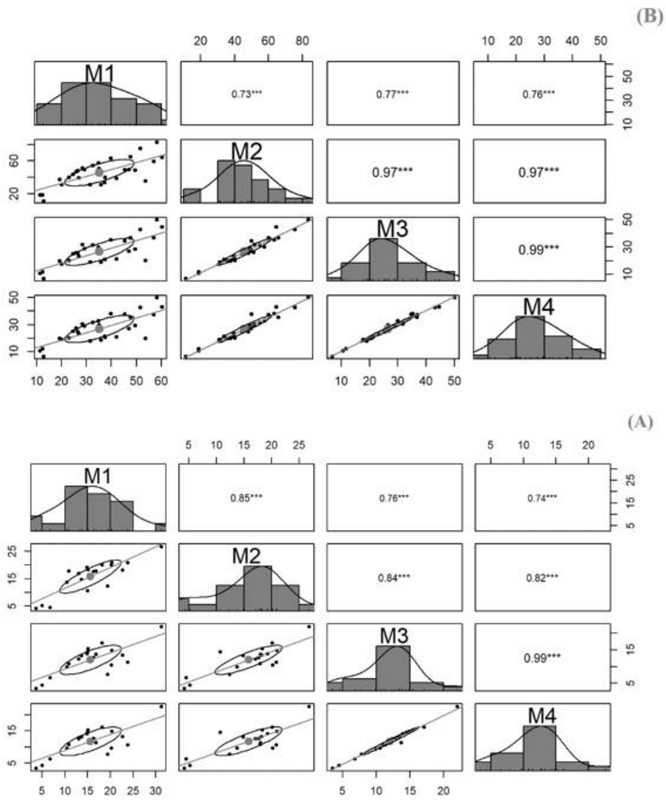

Figure 3. - *Correlation matrix of the leaf area estimated by non-destructive methods in C. officinalis plants planted as a forest stand (A) and by enrichment bands (B).*

Statistical differences between methods

The AF estimated by the four study methods for *C. officinalis leaves* planted by forest stand showed significant differences between methods M1, M2 with M3, M4; while the AF calculated in *C. officinalis plants* planted by enrichment strips showed significant differences between method M1 with the rest of the methods, M2 with the other three methods evaluated, however, no differences were evident between M3 and M4 (Table 1).

Table 1 *- Results of the Friedman non-parametric test applied to leaf area data estimated by non-destructive methods in C. officinalis plants planted as a forest stand and in enrichment strips. Different capital letters for each method indicate significant differences*

Method	**Forest massif**		**Enrichment belts**	
	Sum of ranks	**Cluster**	**Sum of ranks**	**Cluster**
M1	60	TO	87	B
M2	65	TO	113	TO
M3	38	B	47	C
M4	27	B	53	C

DISCUSSION

ImageJ software algorithms M3 and M4 provided the most accurate and consistent estimates of leaf area (LA) for *Cinchona officinalis*, with a very high correlation (r = 0.99) and no significant differences between them, regardless of the planting system evaluated. This behavior is consistent with previous studies in other crops where ImageJ has shown high reliability for morphometric measurements, especially when using manual selection tools and precise scale calibration (Easlon and Bloom 2014; Ferreira *et al.,* 2017). The agreement between M3 and M4 suggests that the choice between the two may be based more on ergonomics and processing time than on differences in accuracy.

ImageJ 's M1 (LeafArea) and M2 methods exhibited significant overestimations of AF, particularly under shaded and humid conditions. This pattern could be explained by the sensitivity of their algorithms to variations in light intensity and the presence of shadows and reflections, factors that affect edge recognition and thresholding in image segmentation (Ahmad *et al.,* 2015; Lee and Lee, 2011). Similar results were reported in *Capsicum. annuum* (Swart *et al.,* 2004) and in fruit species (Kirk *et al., 2009)*, where differences in leaf coloration and light heterogeneity generated biases in the estimation.

The significance of this finding is twofold. On the one hand, it confirms that methodologies based on digital image processing can successfully replace destructive and costly methods, such as planimeters or specialized devices (Confalonieri *et al.*, 2013;

Casa, Upreti, and Pelosi, 2019, which is crucial for threatened species like *C. officinalis*, where leaf preservation is essential to avoid compromising plant vigor. On the other hand, it demonstrates that method selection should consider not only technical precision but also the environmental conditions under which it will be applied, since light and humidity can affect the accuracy of the measurements.

From an applied perspective, the use of M3 and M4 represents an opportunity to establish rapid, low-cost, and reproducible foliar monitoring protocols in conservation and reforestation programs for *C. officinalis*. Reliable foliar measurement is fundamental for evaluating photosynthetic capacity and the physiological state of the plant, as well as for modeling productivity and water requirements (Blanco and Folegatti 2005; Syvertsen, Goñi, and Otero 2003). Its implementation in the field would allow for obtaining time series data to support management and genetic selection decisions.

However, this study has limitations that should be considered. The sample size and the single-stage evaluation restrict the generalizability of the results. Furthermore, the seasonal influence and foliar color variability were not assessed, aspects that have been shown to affect the accuracy of digital measurements (Kirk *et al.,* 2009). Future research should include inter-seasonal comparisons , different planting ages, and a larger number of samples to strengthen the statistical validity and extrapolation of results.

ImageJ 's M3 and M4 methods as the primary tools for estimating arbuscular fluid (AF) in *C. officinalis*, provided they are performed under controlled light and humidity conditions. Their incorporation into monitoring programs will allow for progress toward more efficient management strategies, aligned with the conservation and sustainable use of this emblematic Andean species.

CONCLUSION

ImageJ 's M3 and M4 methods are confirmed as accurate, fast, and low-cost tools for the non-destructive estimation of leaf area in *C. officinalis* , demonstrating superior and more consistent performance than LeafArea (M1) and M2 under the evaluated conditions. Their application in the field can optimize monitoring programs for this endangered species, allowing for the collection of reliable data in short timeframes and with limited resources. Validation is recommended in different development phases and in other ecologically and economically important forest species.

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The authors have participated in the writing of the work and analysis of the documents.