

Leaf area prediction models from growth measurements in Andean blueberry (*Vaccinium meridionale* Swartz) in the nursery

Modelos de predicción de área foliar a partir de mediciones de crecimiento en agraz (*Vaccinium meridionale* Swartz) en vivero

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ABSTRACT

The Andean blueberry is a high-Andean wild fruit species consumed in fresh or processed form that has high potential due to its antioxidant capacity. Leaf area describes the photosynthetic capacity of plants and is employed as a variable in multiple physiological studies; however, in Andean blueberry (*Vaccinium meridionale* Swartz), its direct measurement is costly. The aim of this research was to propose models for estimating the leaf area in young Andean blueberry plants using morphometric variables. In the study, 436 Andean blueberry plants of different ages (10 to 26 months) obtained with different methods of asexual propagation (tissue culture or cuttings) were used. Variables, such as dry weight per organ, leaf area, plant height, number of vegetative shoots and number of leaves, were measured. Simple and multiple regressions were performed and the “weighted least squares” technique was used to meet the regression assumptions. Five models with coefficients of determination (R^2) greater than 0.81 were proposed. Two models were of the multiple type and employed the number of leaves together with the dry weight of leaves or the total dry weight as predictor variables. The other models were linear and used total dry weight, dry weight of leaves or number of leaves as explanatory variables of leaf area; the number of leaves was a particularly interesting variable due to its non-destructive nature. The models presented could be a useful tool for estimating leaf area in future studies in Andean blueberry.

Key words: average leaf area, regression analysis, weighted least squares, Andean species, Ericaceae.

RESUMEN

El agraz es un frutal silvestre altoandino de consumo en fresco o procesado que presenta alto potencial debido a su capacidad antioxidante. El área foliar describe la capacidad fotosintética de las plantas y se emplea como variable en múltiples estudios fisiológicos; no obstante, en agraz (*Vaccinium meridionale* Swartz) su medición directa resulta dispendiosa. El objetivo de esta investigación fue plantear modelos para estimar el área foliar en plantas jóvenes de agraz, empleando variables de tipo morfométrico. Se utilizaron 436 plantas de agraz de diferentes edades (entre 10 y 26 meses) obtenidas a partir de propagación asexual (estacas o cultivo de tejidos). Se midieron variables como peso seco por órgano, área foliar, altura de la planta, número de brotes vegetativos y número de hojas. Se realizaron regresiones simples y múltiples y se utilizó la técnica de “mínimos cuadrados ponderados” para cumplir con los supuestos de la regresión. Se plantearon cinco modelos con coeficientes de determinación (R^2) superiores a 0.81. Dos modelos fueron de tipo múltiple y emplearon el número de hojas junto con el peso seco de hojas o el peso seco total como variables predictoras. Los otros tres modelos fueron de tipo lineal y utilizaron el peso seco total, el peso seco de hojas o número de hojas como variables explicativas del área foliar, siendo el número de hojas particularmente interesante por su carácter no destructivo. Los modelos presentados pueden ser una herramienta útil para estimar el área foliar en futuras investigaciones en agraz.

Palabras clave: área foliar promedio, análisis de regresión, mínimos cuadrados ponderados, especies andinas, Ericaceae.

Introduction

The Andean blueberry (*Vaccinium meridionale* Swartz) (Ericaceae) is a woody shrub that grows spontaneously in the mountainous areas of Colombia, Ecuador, Peru, Venezuela, and Jamaica (Muñoz *et al.*, 2009; Torres *et al.*, 2009; Maldonado Celis *et al.*, 2017). The Andean blueberry plants

have a high domestication potential for fruit consumption in fresh and industrial processing due to the fruit's antioxidant activity and high content of phenolic compounds including anthocyanins, which can exceed those found in commercial species such as blueberries (Bernal *et al.*, 2014; Maldonado Celis *et al.*, 2017; Garzón *et al.*, 2020).

Received for publication: September 30, 2022. Accepted for publication: December 10, 2022.

Doi: 10.15446/agron.colomb.v40n3.105039

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The nursery material corresponds to plants cultivated for subsequent transplanting to the field (Singh *et al.*, 2017); this is decisive in the quality and productivity of crops (Sánchez-Aguilar *et al.*, 2016), so multiple studies focus on this phase. In Andean blueberry propagated from seeds, the nursery stage of growth ends after approximately 26 months (Torres *et al.*, 2012). For asexually propagated Andean blueberry, few reports indicate the duration of the nursery period. According to González *et al.* (2018), a duration of 5 months is an adequate period for a rooted cutting of Andean blueberry to reach a size of around 20 cm in height and be suitable for transplanting to the field. Medina-Cano *et al.* (2015) report a period between 15 and 18 months from preparing the cuttings to their transplanting to the final site. Due to the taxonomic relationship of Andean blueberry with blueberry (*V. corymbosum*), reports for early growth of blueberry may be adjusted to Andean blueberry. Generally, in blueberries propagated from cuttings or by tissue culture, the nursery stage lasts from 18 to 24 months; after this period the plants are considered ready to be transplanted to the final site (Bryla & Machado, 2011; Bryla & Strik, 2015). In young plants of Andean blueberry, initial growth is characterized by extensive leaf formation. Vegetative growth progresses through formation and differentiation of axillary buds, followed by expansion and development of leaf blades. Finally, young leafy shoots are developed, characterized by the presence of leaves, prominent elongation of branches and leaf expansion, and formation of new axillary buds that can be differentiated into vegetative or reproductive organs (Medina-Cano *et al.*, 2019).

The leaf area (LA) quantifies the total leaf surface that a plant possesses; this variable is of high importance in research in biology, plant physiology, agronomy, and ecology (Cabezas-Gutiérrez *et al.*, 2009; Weraduwege *et al.*, 2015). The LA is used as a response or predictor variable in processes such as light interception for photosynthesis and transpiration for efficient water use, and as an indicator of growth and productivity (Kucharik *et al.*, 1998; Montoya-Restrepo *et al.*, 2017). At the plant community level, LA is the basis for estimating light interception indicators, such as the leaf area index (LAI) used in function-adjusted growth analysis (Cabezas-Gutiérrez *et al.*, 2009; Benedetto & Tognetti, 2016; Hunt, 2017).

The methods to estimate LA in plants can be classified into direct and indirect groups (Yan *et al.*, 2019). The direct or destructive methods limit the possibility of measuring the same individual as a function of time in growth studies and require intensive labor, as they involve removing the leaves

of a plant to measure them with different techniques. Direct LA measurement can be done with LI-3000C portable leaf area meter (LI-COR, Lincoln, NE, USA) or the Portable Laser Leaf Area Meter (CID Bio-Science, Camas, WA, USA). The use of these meters is limited because of their cost. As an alternative, the use of images is a strategy that allows estimating values from the use of image processing software such as ImageJ (Rasband, 1997) used in various studies (Schneider *et al.*, 2012; Easlon & Bloom, 2014; Milić *et al.*, 2018; Suarez Salazar *et al.*, 2018; Cosmulescu *et al.*, 2020; Wakui & Kudo, 2021), Adobe Photoshop (Adobe Systems Incorporated, 1990), and AutoCAD (Autodesk, 1982), which are based on the use of a scale and the manual segmentation of images to differentiate the background of leaves.

The LA estimation is useful when the number of experimental units or observations is small or the same experimental unit is monitored, such as in perennial or timber crops, or where direct measurement of LA is costly (Montoya *et al.*, 2017). Indirect methods obtain an approximation to LA from mathematical models based on non-destructive morphometric measurements and indicators of growth including: i) the ratio of length and width of leaves, number of leaves, and combination of these variables (NeSmith, 1991; Igathinathane *et al.*, 2006; Fallovo *et al.*, 2008; Spann & Heerema, 2010; Celik *et al.*, 2011; Cabezas-Gutiérrez & Peña-Baracaldo, 2012; Suárez Salazar *et al.*, 2018; León-Burgos *et al.*, 2022), and ii) models or relationships with other variables such as leaf dry weight (Akram-Ghaderi & Soltani, 2007; Huang *et al.*, 2019) among other variables. One of the limitations in using mathematical models is their specificity at the level of variety, cultivar, phenological stages (Pire & Venezuela, 1995) and stratum in the canopy (Brito *et al.*, 2007). This generally requires direct methodologies for the model validation (Yan *et al.*, 2019), ignoring, in some cases, the use of other growth variables related to LA that could increase the precision of the LA estimation.

The Andean blueberry has small, simple, alternate, coriaceous, lanceolate or oblong-lanceolate leaves, with a serrated margin, cuneate leaf base and acute apex (Ehlenfeldt *et al.*, 2022). In blueberries, structural traits of leaves and stems can correlate with yield parameters (Barai *et al.*, 2022), while, for the Andean blueberry, no studies have been carried out in this regard. Additionally, leaves can be one of the by-products of the Andean blueberry cultivation (Zapata-Vahos *et al.*, 2015) since they have a high content of polyphenols and other substances of nutraceutical and medicinal importance (Borda-Yepes *et al.*, 2019). Direct measurement of LA in Andean blueberry plants is difficult

due to the high number of leaves and their small size (Ehlenfeldt *et al.*, 2022), resulting in a slow and meticulous procedure. In other *Vaccinium* species (*V. corymbosum*, *V. virgatum*, and others), models have been developed for the measurement of leaf area. However, these species are deciduous, while the Andean blueberry is an evergreen perennial plant; therefore, it is important to develop specific models for the Andean blueberry that allow a better estimation of this variable given the difficulty of direct LA measurement and the scarcity of alternative methods for estimating its LA. The purpose of this research was to establish five models to estimate LA in young Andean blueberry plants. These models employed various growth variables and used experimental and statistical validation.

Materials and methods

Plant material and experiment establishment

The experiment was carried out between 2019 and 2021 under greenhouse conditions at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogotá, at an altitude of 2,550 m a.s.l., with average air temperature $18.6 \pm 10^\circ\text{C}$, average relative humidity $66 \pm 26\%$ (ELMA DT-171 datalogger, Elma instruments, Ryttermarken, Denmark). In total, 436 Andean blueberry plants propagated asexually were used in the vegetative stage of growth, with ages from 10 to 26 months (Tab. 1). The leaf area (LA) of 54,492 leaves was measured. The first group of plants was obtained by rooting of apical cuttings of wild plants from the municipalities of Guachetá, Cundinamarca (2800 m a.s.l., $5^\circ 26' 71.8''$ N and $73^\circ 42' 09.2''$ W) and San Miguel de Sema, Boyacá (2572 m a.s.l., $5^\circ 36' 09.3''$ N and $73^\circ 45' 23.8''$ W); rooted cuttings were planted in 2.0 L plastic pots with a substrate (3:1:0.3 v/v/v): sand + perlite + Klasmann® peat without nutrients. The second and third groups were plants propagated *in vitro* by proliferation of axillary buds in a commercial nursery in the municipality of Rionegro, Antioquia (24°C , 80% relative humidity, 2800 m a.s.l.); the plants were planted in 2.6 L plastic pots with sand + Klasmann® peat without nutrients (5:1 v/v). Phytosanitary management was carried out preventively, with monthly application of a garlic-chili based pest repellent (70 g L^{-1}) (Alisin® - Sáfer Agrobiológicos S.A.S), and a chlorothalonil-based protective fungicide (720 g L^{-1}), both at a dosage of 1 ml L^{-1} .

Nitrogen and phosphorous fertilizations were carried out with doses between 0 to 2.7 and 0 to 1.3 g/plant per 5

months, respectively. The range of fertilization with N and P varied because these plants were the object of another fertilization trial with these mineral nutrients. The rest of the fertilization plan was carried out in the same proportion for all plants ($1.25 \text{ K}_2\text{O}$, 0.73 CaO , $1.08 \text{ MgO g/plant per 5 months}$ and 18.15 Fe , 12.45 Mn , $6.21 \text{ H}_3\text{B}_2\text{O}_3$, 0.70 Zn , and $0.70 \text{ Cu mg/plant per 5 months}$). The total fertilizer dose was divided into 4 applications realized at 3, 8, 12, and 16 weeks after transplanting the plants into plastic pots. Fertilizer sources were: urea ($\text{CO}(\text{NH}_2)_2$), calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), di-ammonium phosphate ($(\text{NH}_4)_2\text{HPO}_4$), triple super phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot x\text{H}_2\text{O}$), potassium sulfate (K_2SO_4), calcium sulfate (CaSO_4), magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot x7\text{H}_2\text{O}$), iron sulfate (FeSO_4), boric acid (H_3BO_3), zinc sulfate (ZnSO_4), copper sulfate (CuSO_4), and manganese sulfate (MnSO_4). Fertilization doses were established in accordance with the research by González *et al.* (2018) and studies conducted on plants of the genus *Vaccinium* (Percival & Privé, 2002; Jeliaskova & Percival, 2003; Percival *et al.*, 2003; Nestby *et al.*, 2014; Maqbool *et al.*, 2017) and a pre-assay of this research.

TABLE 1. Characteristics of the Andean blueberry plants used in the experiment.

Propagation type	Group	Age (months)	Number of plants
Cuttings	1	10	50
		14	70
	2	16	68
		18	68
	3	22	72
		24	63
<i>In vitro</i>		26	45

Evaluated variables

A descriptive analysis of the average leaf area (ALA) calculated from the total LA of each plant divided by the number of leaves was carried out to compare the groups of plants. Regressions were made from non-destructive variables such as height (H), measured from the base of the stem to the last apical vegetative shoot, number of vegetative shoots (NS), which, in the case of *V. meridionale*, are reddish in color due to the presence of anthocyanin pigments, and number of leaves (NL) of each plant (Medina-Cano *et al.*, 2015; González *et al.*, 2018). Similarly, regressions were performed for destructive variables such as dry weight per organ (DW) and LA, the latter as an explanatory variable.

To determine the total DW per plant, the plants were separated into roots, stems, and leaves and dried in a muffle

oven at 75°C until constant weight. Subsequently, the following were determined: the root dry weight (RDW), leaf dry weight (LDW), stem dry weight (SDW) of plants propagated by cuttings, dry weight of the branches originating from stems in plants propagated *in vitro* (BSDW) and the total plant dry weight (TPDW) by adding the weight of all organs (Becerra *et al.*, 2022). The leaf area (LA) of each plant was determined from digital image processing using the software ImageJ® v. 1.52 (Schneider *et al.*, 2012). The photographs were taken with a mobile phone 25 MP camera at a height of 60 cm.

Statistical analysis

Statistical analysis was performed using the statistical software R (version 3.5.1.) and the respective libraries according to the statistical procedures described below. An exploratory analysis was carried out from the average LA of a single leaf to compare the groups of plants according to age. A descriptive analysis was performed from scatter diagrams to identify patterns in the relationships of variables. Pearson correlations were used to identify and quantify the linear relationship between variables with respect to LA. The plots were made using the “ggplot2” library (Wickham, 2016) and the correlation matrix was obtained with the “GGally” library extension (Schloerke *et al.*, 2021).

From the variables with the highest correlation, various simple or multiple linear regression models were explored. In order to establish or propose a model that involves only non-destructive variables, models were tested with the predictor variables NL, H and NS, and LA as a response. Compliance with the regression assumptions was evaluated for all models. As morphometric variables and performance indicators are usually highly correlated, multicollinearity was evaluated from the variance inflation factor (VIF) with the “DescTools” library (Signorell, 2018). Similarly, linearity was studied using Pearson’s correlation and residual normality using the Kolmogorov-Smirnov normality test, both from the “stats” library (R Core Team, 2022). Heteroscedasticity was evaluated using the “Non-constant Variance Score Test” from the “car” library (Fox & Weisberg, 2019) and the presence of influential data was evaluated using the Cook distance criterion. In cases where some of the regression assumptions were not met, the weighted least squares technique was used (PennState, 2021). Finally, the models that met the assumptions were compared based on their predictions using the root mean square error (Root Mean Square Error, RMSE).

Results and discussion

Determination of the average leaf area (ALA) has been used to estimate the total leaf area in commercial blueberry plants and wild blueberries (Fallico *et al.*, 2008; Celik *et al.*, 2011; Lancheros, 2012; Becerra *et al.*, 2022) and perennial crops such as cherry (*Prunus avium*) and coffee (*Coffea arabica*) (Cittadini & Peri, 2006; Taugourdeau *et al.*, 2014). For the consolidated database of the Andean blueberry, ALA varied between 0.03 cm² (first quartile) and 1.85 cm² (fourth quartile), with a median of 0.94 cm² and a mean of 0.93 cm² (Fig. 1). However, the mean ALA presented differences between the study groups, being 0.44 cm² for group 1 in which all plants were propagated by cuttings, 0.92 cm² for group 2 and 1.07 cm² for group 3; plants in these last two groups were propagated *in vitro*.

Between the groups, differences were observed in the ALA related to the type of propagation (Fig. 1). The plants propagated by cuttings presented lower ALA (group 1: 0.45 ± 0.27 cm²) compared to plants propagated *in vitro* (group 2: 0.92 ± 0.26 cm², group 3: 1.03 ± 0.22 cm²). The ALA for the group of plants propagated by cuttings coincides with the range of 0.1 to 0.3 cm² described by Lancheros (2012) for plants propagated by seeds.

Variations in ALA were reported in wild and domesticated blueberries depending on the method of propagation, with tall and medium-tall blueberry plants obtained *in vitro* growing faster and having a greater number of stems, more lateral branches, and a greater number of shoots as well as higher canopy area and plant dry weight compared to plants obtained by stem cuttings (Marino *et al.*, 2014). Blueberry (*V. corymbosum* L.) plants micropropagated *in vitro* produced more and longer shoots than plants obtained from semi-woody cuttings (Litwinczuk *et al.*, 2005). This coincides with the present research, which observed shorter primary branches and shorter internodes in plants propagated by cuttings as compared to longer primary branches of *in vitro* propagated plants. Additionally, *in vitro* propagated plants of Andean blueberry had a planophyllous, almost runner-type branch formation, which promotes higher leaf production (Fig. 2). The higher number of shoots per plant in the *in vitro* propagated plants could also be due to the differences in plant age or size between the two propagation methods during this study.

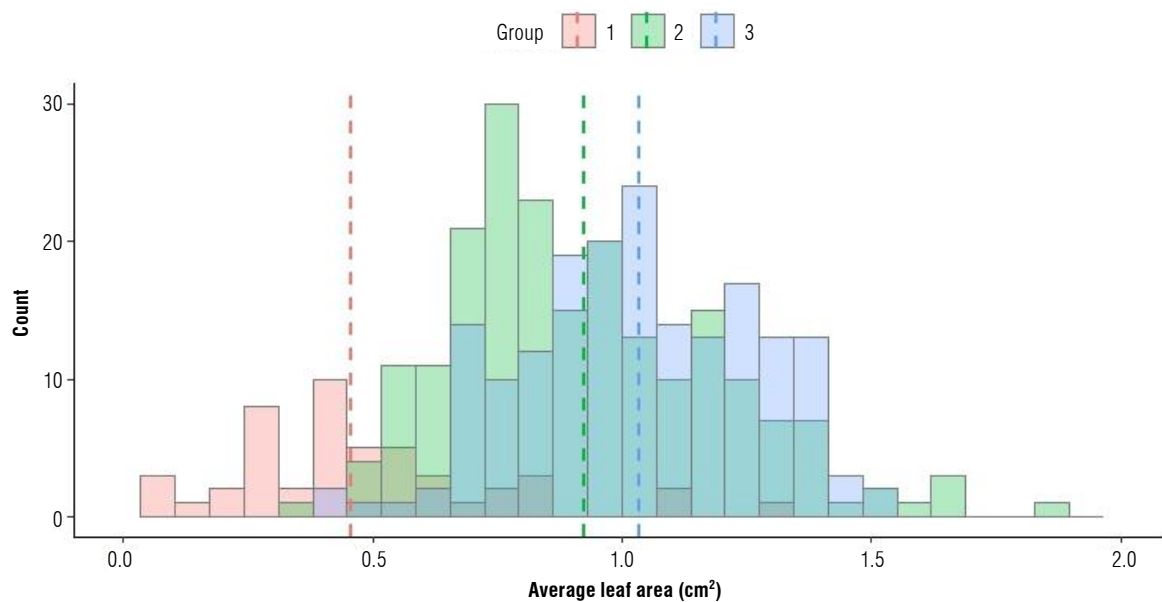


FIGURE 1. Histogram of the average leaf area for each of the Andean blueberry (*Vaccinium meridionale* Swartz) plant groups. Group 1: Young plants 10 months old (red) propagated by cuttings. Group 2: young plants 14 to 18 months old (blue) and group 3: young plants 22 to 26 months old (green) propagated in vitro. Dotted lines represent mean of each group. $n=436$.

The ALA differed according to age; plants 10 (0.45 ± 0.27 cm²) and 14 (0.75 ± 0.18 cm²) month-old had lower ALA values, while plants 16 (0.99 ± 0.24 cm²), 18 (1.03 ± 0.27 cm²), 22 (0.99 ± 0.23 cm²), 24 (1.01 ± 0.22 cm²), and 26 (1.13 ± 0.20 cm²) month-old had a similar average ALA value (Fig. 3).

Most of the variables evaluated presented linear relationships with LA, except for plant height (H), number of vegetative shoots (NS), and root dry weight (RDW) (Fig. 4). Although in different plant species a linear relationship between LA and H has been reported (Calvo-Alvarado *et al.*, 2008; Fanourakis *et al.*, 2014), in Andean blueberry this relationship was not observed, apparently, due to the relatively low rate of growth of this species.

For the consolidated data, LA presented the highest Pearson correlation values with leaf dry weight (LDW) (0.95), total plant dry weight (TPDW) (0.95) and number of leaves (NL) (0.92) variables. The NL relationship with LA differed by groups, being similar between the groups of *in vitro* propagated plants (group 2: 0.88, group 3: 0.92) and higher than the young plants propagated by cuttings (group 1: 0.60), which suggests that the relationship between LA and NL differs according to the type of propagation (Fig. 2). Considering that the variables LDW, TPDW and NL presented the highest correlation values according to the methodology proposed by Mukaka (2012) and that the correlation between the groups was similar, models for leaf

area prediction were proposed based on LDW, TPDW and NL, while the other variables were discarded.

The linear and multiple models initially proposed failed to meet some of the assumptions, but two of the multiple and three of the linear models (Tab. 2) from their weighting with the errors, using the weighted least squares technique, did meet the assumption. This technique is useful for observations that present non-constant variances in the errors (PennState, 2021) and was employed by Panigrahi and Sankar Das (2020), where they used this method to improve the remote estimation of the leaf area index and canopy water content in rice plants under water stress.

The multiple models (1 and 2) described in Table 3 had higher determination coefficients and a lower error, which shows their high capacity to estimate LA; however, their use implies the measurement of two variables, which depending on the case were LDW and NL or TPDW and NL. Considering the parsimony criterion, the use of linear models can be an interesting alternative, since this requires a single variable. Although models 3 and 4 use a destructive variable, it is experimentally easier and more accessible to weigh the plant organ than to make a direct measurement of LA. The Andean blueberry has numerous small-sized leaves (Ehlenfeldt *et al.*, 2022), which makes it difficult to directly measure the LA or to perform other types of non-destructive measurements such as leaf length or width (Lancheros, 2012).

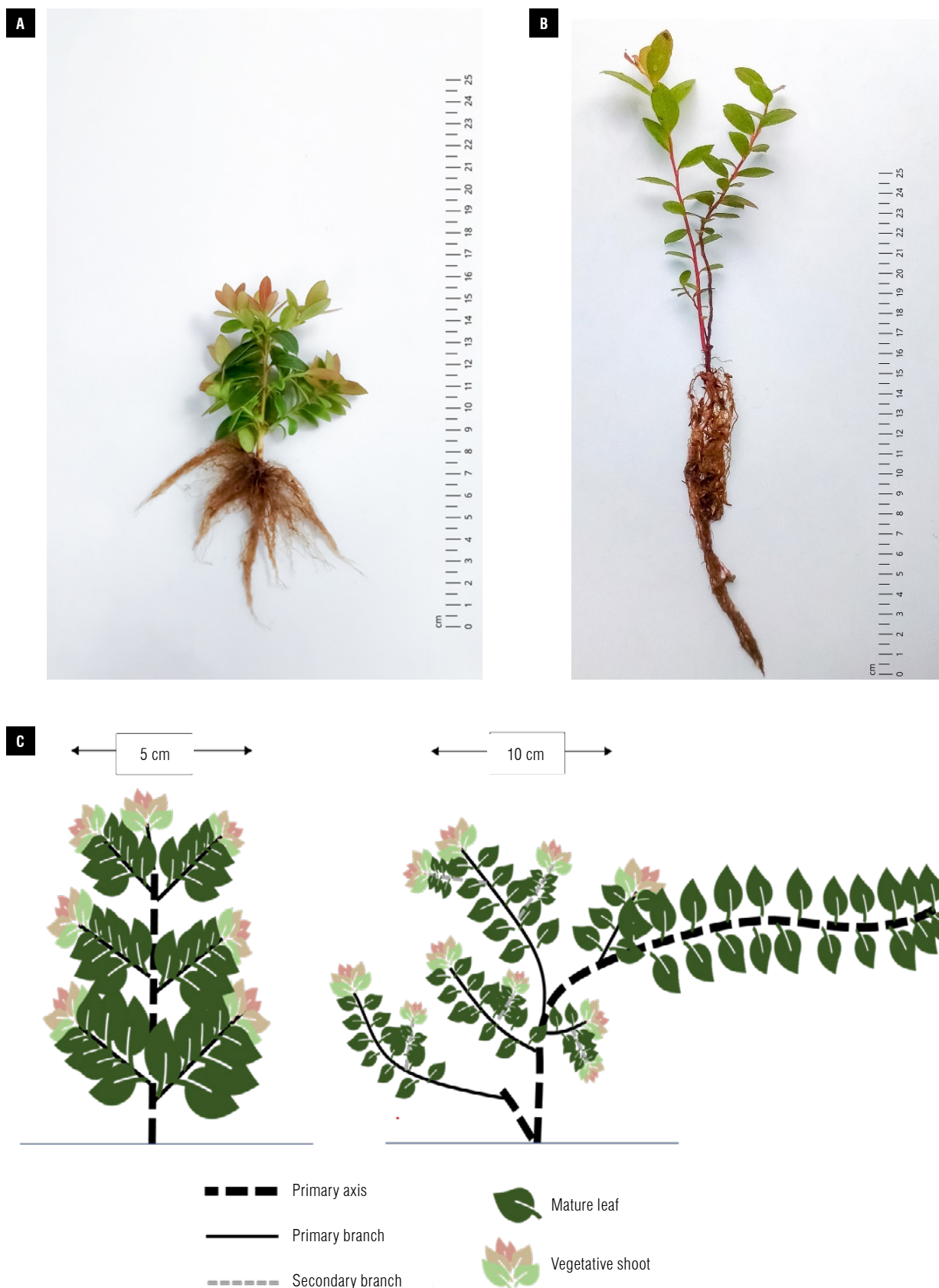


FIGURE 2. Photographs (A and B) and schematic representation (C) of the architecture of young Andean blueberry (*Vaccinium meridionale* Swartz) propagated from cuttings (A) and *in vitro* (B). The boxes in Fig. 2C indicate the scale in cm.

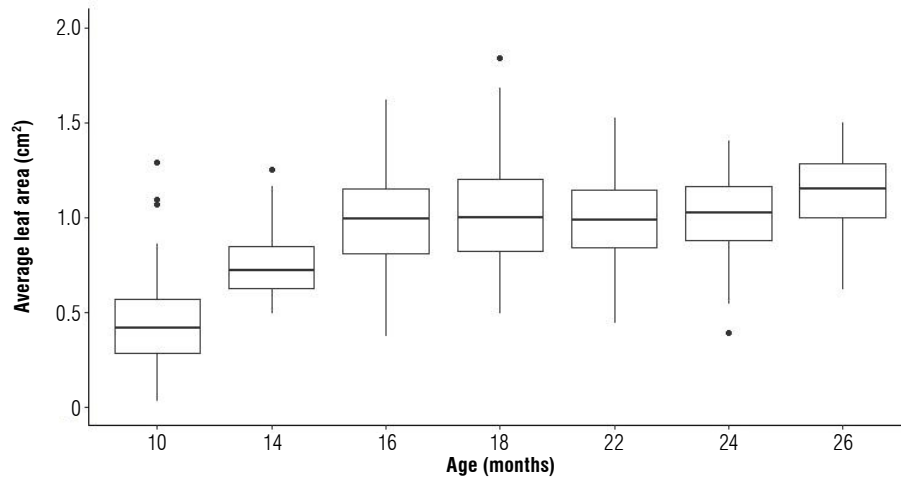


FIGURE 3. Box-and-whisker plot for average leaf area (ALA) in young Andean blueberry (*Vaccinium meridionale* Swartz) plants according to their age. Plants 10 months old propagated from cuttings and plants 14 to 26 months old propagated *in vitro*. n=436.

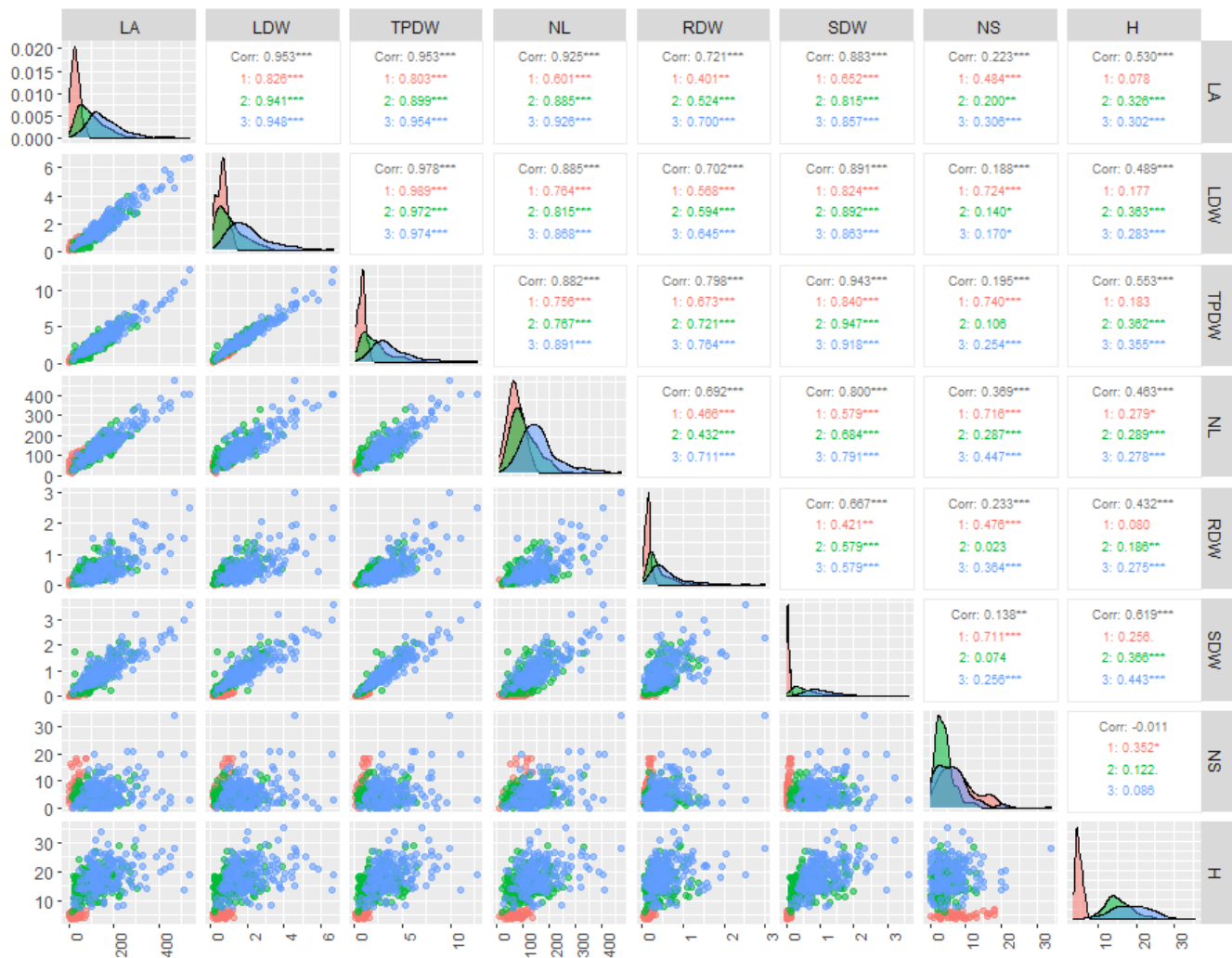


FIGURE 4. Dispersion, distribution, and Pearson correlation by pairs for the variables leaf area (LA), leaf dry weight (LDW), total plant dry weight (TPDW), number of leaves (NL), root dry weight (RDW), stem dry weight (SDW), number of shoots (NS), and height (H) of young plants of Andean blueberry (*Vaccinium meridionale* Swartz). Group 1: young plants 10 months old (red) propagated by cuttings. Group 2: young plants 14 to 18 months old (blue) and group 3: young plants 22 to 26 months old (green) propagated *in vitro* and the consolidation of these groups (black). n=436.

TABLE 2. Regression models with the best fit for estimating leaf area in young plants of the Andean blueberry.

N°	Model	R ²	R ² adjusted	RMSE
1	LA = (28.392 × TPDW) + (0.465 × NL) -12.752	0.9182	0.9178	21.740
2	LA = (51.119 × LDW) + (0.457 × NL) -10.766	0.8104	0.8100	21.753
3	LA = (77.870 × LDW) + 7.191	0.8735	0.8732	26.579
4	LA = (43.474 × TPDW) + 5.229	0.8733	0.8730	26.791
5	LA = (1.117 × NL) - 18.730	0.8104	0.8100	33.702

LA: Leaf area per plant, cm²; LDW: Leaf dry weight of an individual plant, mg; TPDW: Total plant dry weight, mg; NL: Number of leaves per plant. R²: Coefficient of determination; R² adj: Coefficient of determination adjusted for multiple variables; RMSE: Root Mean Square Error.

The leaf dry weight (LDW) is considered the most appropriate criterion to estimate plant photosynthetic assimilation, as it is closely related to the LA (Taiz *et al.*, 2018). This is in agreement with Akram-Ghaderi and Soltani (2007) who showed that, for cotton, the power equation best described the relationship between LA and LDW. Finally, model 5 is an LA estimation alternative that uses a non-destructive variable, such as the number of leaves (NL). Similar studies in perennial crops, such as grapevines (Lopes & Pinto, 2005) and coffee (Montoya *et al.*, 2017), show that models based on the NL result in an easy-to-measure and economical alternative to estimate LA. This has also been reported in semi-annual crops such as corn by Dwyer and Stewart (1986) and Elings (2000); the latter author highlighting that this relationship is true if the average LA of a single leaf is known and the plant has leaves of similar size, as is the case of the Andean blueberry (Ehlenfeldt *et al.*, 2022).

Conclusions

The differences found in plant architecture and the average leaf area in young Andean blueberry plants depending on the propagation method (by cuttings or *in vitro*) must be considered for the planning of agronomic practices, such as pruning, studying the canopy architecture (plant density) and frequency of agronomic labors. This research proposes five models for an adequate estimation of leaf area that used destructive and non-destructive variables, and their combination. The adjustment of the models using weighted least squares fulfilled the assumptions for some of the proposed models. The use of the models developed in this research, especially the one that uses number of leaves (a non-destructive variable), could facilitate future studies for the determination of leaf area in this species.

Acknowledgments

The authors express their gratitude to the Faculty of Agricultural Sciences, Universidad Nacional de Colombia (Bogotá), especially to the Center for Research and Rural

Extension (Centro de Investigación y Extension Rural - CIER), for the partial financing of the research within the framework of the project “Support for registered research groups that contribute to strengthening research in the Faculty of Agricultural Sciences of the Bogota campus” (QUIPU No. 207010012262) through the Horticulture research group of Minciencias. We are also grateful to the producers of the Andean blueberry from the municipalities of Guachetá (Cundinamarca) and San Miguel de Sema (Boyacá), who permitted the collection of plant cuttings from their properties and to C.I. Agroberries nursery located in Rionegro (Antioquia), who supplied *in vitro* propagated plants.

Author's contributions

MVM: conceptualization, research, writing - original draft, visualization, writing, and editing. PLP: conceptualization, visualization, statistical analysis, writing, and editing. ED: conceptualization, statistical analysis, writing, and editing. LPMF: supervision, writing, and editing. SM: supervision, project administration, writing, and editing. All authors have read and approved the final version of the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Literature cited

- Adobe Systems Incorporated. (1990). *Adobe Photoshop*. Microsoft Windows.
- Autodesk. (1982). *AutoCAD*. Microsoft Windows.
- Akram-Ghaderi, F., & Soltani, A. (2007). Leaf area relationships to plant vegetative characteristics in cotton (*Gossypium hirsutum* L.) grown in a temperate sub-humid environment. *International Journal of Plant Production*, 1(1), 63–71.
- Barai, K., Calderwood, L., Wallhead, M., Vanhanen, H., Hall, B., Drummond, F., & Zhang, Y. J. (2022). High variation in yield among wild blueberry genotypes: Can yield be predicted by leaf and stem functional traits? *Agronomy*, 12(3), Article 617. <https://doi.org/10.3390/agronomy12030617>

- Becerra, A. D., Quevedo-Rubiano, S., Magnitskiy, S., & Lancheros, H. O. (2022). Morphological responses of Andean blueberry (*Vaccinium meridionale* Swartz) plants growing in three environments at different altitudes. *Revista Colombiana de Ciencias Hortícolas*, 16(3), Article e15034.
- Benedetto, D., & Togmetti, J. (2016). Técnicas de análisis de crecimiento de plantas: su aplicación a cultivos intensivos. *Revista de Investigaciones Agropecuarias*, 42(3), 258–282.
- Bernal, L. J., Melo, L. A., & Díaz Moreno, C. (2014). Evaluation of the antioxidant properties and aromatic profile during maturation of the blackberry (*Rubus glaucus* Benth) and the bilberry (*Vaccinium meridionale* Swartz). *Revista Facultad Nacional de Agronomía Medellín*, 67(1), 7209–7218. <https://doi.org/10.15446/rfnam.v67n1.42649>
- Borda-Yepes, V. H., Chejne, F., Daza-Olivella, L., Alzate-Arbelaes, A. F., Rojano, B. A., & Raghavan, V. G. S. (2018). Effect of microwave and infrared drying over polyphenol content in *Vaccinium meridionale* (Swartz) dry leaves. *Journal of Food Process Engineering*, 42(1), Article e12939. <https://doi.org/10.1111/jfpe.12939>
- Brito, E., Romero, E. R., Casen, S. D., Alonso, L. G., & Digonzelli, P. A. (2007). Métodos no destructivos de estimación del área foliar por tallo en la variedad LCP 85-384 de caña de azúcar. *Revista Industrial y Agrícola de Tucumán*, 84(2), 29–32.
- Bryla, D. R., & Machado, R. M. A. (2011). Comparative effects of nitrogen fertigation and granular fertilizer application on growth and availability of soil nitrogen during establishment of highbush blueberry. *Frontiers in Plant Science*, 2, Article 46. <https://doi.org/10.3389/fpls.2011.00046>
- Bryla, D. R., & Strik, B. C. (2015). Nutrient requirements, leaf tissue standards, and new options for fertigation of northern highbush blueberry. *HortTechnology*, 25(4), 464–470. <https://doi.org/10.21273/horttech.25.4.464>
- Cabezas-Gutiérrez, M., & Peña-Baracaldo, F. (2012). Estimación del área foliar del arándano (*Vaccinium corymbosum*) por medio de un método no destructivo. *Revista U.D.C.A Actualidad & Divulgación Científica*, 15(2), 373–379. <https://doi.org/10.31910/rudca.v15.n2.2012.837>
- Cabezas-Gutiérrez, M., Peña, F., Duarte, H. W., Colorado, J. F., & Lora Silva, R. (2009). Un modelo para la estimación del área foliar en tres especies forestales de forma no destructiva. *Revista U.D.C.A Actualidad & Divulgación Científica*, 12(1), 121–130. <https://doi.org/10.31910/rudca.v12.n1.2009.648>
- Calvo-Alvarado, J. C., McDowell, N. G., & Waring, R. H. (2008). Allometric relationships predicting foliar biomass and leaf area:sapwood area ratio from tree height in five Costa Rican rain forest species. *Tree Physiology*, 28(11), 1601–1608. <https://doi.org/10.1093/treephys/28.11.1601>
- Celik, H., Serhat-Odabas, M., & Odabas, F. (2011). Leaf area prediction models for highbush blueberries (*Vaccinium corymbosum* L.) from linear measurement. *Journal of Microbiology, Biotechnology and Food Sciences*, 33(1), 16–21.
- Cittadini, E. D., & Peri, P. L. (2006). Estimation of leaf area in sweet cherry using a non-destructive method. *Revista de Investigaciones Agropecuarias*, 35(1), 143–150.
- Cosmulescu, S., Scricieiu, F., & Manda, M. (2020). Determination of leaf characteristics in different medlar genotypes using the ImageJ program. *Horticultural Science*, 47(2), 117–121. <https://doi.org/10.17221/97/2019-HORTSCI>
- Easlon, H. M., & Bloom, A. J. (2014). Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Sciences*, 2(7), Article 1400033. <https://doi.org/10.3732/apps.1400033>
- Ehlenfeldt, M. K., Polashock, J. J., Rowland, L. J., Ogden, E., & Luteyn, J. L. (2022). Fertile intersectional hybrids of 4x Andean blueberry (*Vaccinium meridionale*) and 2x lingonberry (*V. vitis-idaea*). *HortScience*, 57(4), 525–531. <https://doi.org/10.21273/hortsci16285-21>
- Falovo, C., Cristofori, V., De-Gyves, E. M., Rivera, C. M., Rea, R., Fanasca, S., Bignami, C., Sassine, Y., & Roupheal, Y. (2008). Leaf area estimation model for small fruits from linear measurements. *HortScience*, 43(7), 2263–2267. <https://doi.org/10.21273/hortsci.43.7.2263>
- Fanourakis, D., Briesse, C., Max, J. F. J., Kleinen, S., Putz, A., Fiorani, F., Ulbrich, A., & Schurr, U. (2014). Rapid determination of leaf area and plant height by using light curtain arrays in four species with contrasting shoot architecture. *Plant Methods*, 10(1), 1–11. <https://doi.org/10.1186/1746-4811-10-9>
- Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Sage, Thousand Oaks CA. <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>
- Garzón, G. A., Soto, C. Y., López-R, M., Riedl, K. M., Browmiller, C. R., & Howard, L. (2020). Phenolic profile, in vitro antimicrobial activity and antioxidant capacity of *Vaccinium meridionale* Swartz pomace. *Heliyon*, 6(5), Article e03845. <https://doi.org/10.1016/j.heliyon.2020.e03845>
- González, L. K., Rugeles, L. N., & Magnitskiy, S. (2018). Effect of different sources of nitrogen on the vegetative growth of Andean blueberry (*Vaccinium meridionale* Swartz). *Agronomía Colombiana*, 36(1), 58–67. <https://doi.org/10.15446/agron.colomb.v36n1.69304>
- Huang, W., Ratkowsky, D. A., Hui, C., Wang, P., Su, J., & Shi, P. (2019). Leaf fresh weight versus dry weight: Which is better for describing the scaling relationship between leaf biomass and leaf area for broad-leaved plants? *Forests*, 10(3), Article 256. <https://doi.org/10.3390/f10030256>
- Hunt, R. (2017). Growth analysis, Individual plants. In B. Thomas, B. G. Murray, & D. J. Murphy (Eds.). *Encyclopedia of applied plant sciences* (2nd ed., Vol. 1, pp. 421–429). Academic Press.
- Igathinathane, C., Prakash, V. S. S., Padma, U., Babu, G. R., & Womac, A. R. (2006). Interactive computer software development for leaf area measurement. *Computers and Electronics in Agriculture*, 51(1–2), 1–16. <https://doi.org/10.1016/j.compag.2005.10.003>
- Jeliazkova, E. A., & Percival, D. C. (2003). N and P fertilizers, some growth variables, and mycorrhizae in wild blueberry (*Vaccinium angustifolium*). *Acta Horticulturae*, 626, 297–304. <https://doi.org/10.17660/actahortic.2003.626.41>
- Kassambara, A. (2020). Factoextra: Extract and visualize the results of multivariate data analyses. R package versión 1.0.7. <https://CRAN.R-project.org/package=factoextra>
- Kucharik, C. J., Norman, J. M., & Gower, S. T. (1998). Measurements of branch area and adjusting leaf area index indirect measurements. *Agricultural and Forest Meteorology*, 91(1–2), 69–88. [https://doi.org/10.1016/S0168-1923\(98\)00064-1](https://doi.org/10.1016/S0168-1923(98)00064-1)

- Lancheros, H. (2012). Caracterización de las micorrizas nativas en agraz *Vaccinium meridionale* Swartz y evaluación de su efecto sobre el crecimiento plantular [MSc Thesis, Universidad Nacional de Colombia]. <https://repositorio.unal.edu.co/handle/unal/12135>
- León-Burgos, A. F., Ramírez, C., Rendón Sáenz, J. R., Imbach-Quinchua, L. C., Unigarro-Muñoz, C. A., & Balaguera-López, H. E. (2022). Fitting growth curves of coffee plants in the nursery stage of growth: A functional approach. *Agronomía Colombiana*, 40(3), 344–353. <https://doi.org/10.15446/agron.colomb.v40n3.101333>
- Litwinczuk, W., Szregorz, G., & Wrona, D. (2005). Field performance of highbush blueberries (*Vaccinium x corymbosum* L.) cv. “Herbert” propagated by cuttings and tissue culture. *Scientia Horticulturae*, 106, 162–169. <https://doi.org/10.1016/j.scienta.2005.02.025>
- Lopes, C., & Pinto, P. A. (2005). Easy and accurate estimation of grapevine leaf area with simple mathematical models. *Vitis - Journal of Grapevine Research*, 44(2), 55–61.
- Maldonado Celis, M. E., Franco Tobón, Y. N., Agudelo, C., Arango, S. S., & Rojano, B. (2017). Andean berry (*Vaccinium meridionale* Swartz). In E. M. Yahia (Ed.), *Fruit and vegetable phytochemicals: Chemistry and human health* (2nd ed., pp. 869–881). <https://doi.org/10.1002/9781119158042.ch40>
- Marino, S. R., Williamson, J. G., & Olmstead, J. W. (2014). Vegetative growth of three southern highbush blueberry cultivars obtained from micropropagation and softwood cuttings in two Florida locations. *HortScience*, 49(5), 556–561. <https://doi.org/10.21273/hortsci.49.5.556>
- Maqbool, R., Percival, D., Zaman, Q., Astatkie, T., Adl, S., & Buszard, D. (2017). Leaf nutrients ranges and berry yield optimization in response to soil-applied nitrogen, phosphorus and potassium in wild blueberry (*Vaccinium angustifolium* Ait.). *European Journal of Horticultural Science*, 82(4), 166–179. <https://doi.org/10.17660/eJHS.2017/82.4.2>
- Medina Cano, C. I., Lobo Arias, M., Castaño Colorado, Á. A., & Cardona, L. E. (2015). Análisis del desarrollo de plantas de mortiño (*Vaccinium meridionale* Swartz.) bajo dos sistemas de propagación: clonal y sexual. *Ciencia & Tecnología Agropecuaria*, 16(1), Article 390. https://doi.org/10.21930/rcta.voll6_num1_art390
- Medina Cano, C. I., Martínez Bustamante, E., & López Orozco, C. A. (2019). Phenological scale for the mortiño or agraz (*Vaccinium meridionale* Swartz) in the high Colombian Andean area. *Revista Facultad Nacional de Agronomía Medellín*, 72(3), 8897–8908. <https://doi.org/10.15446/rfnam.v72n3.74460>
- Milić, B., Tarlanović, J., Keserović, Z., Magazin, N., Miodragović, M., & Popara, G. (2018). Bioregulators can improve fruit size, yield and plant growth of northern highbush blueberry (*Vaccinium corymbosum* L.). *Scientia Horticulturae*, 235, 214–220. <https://doi.org/10.1016/j.scienta.2018.03.004>
- Montoya Restrepo, E. C., Hernández Arredondo, J. D., Unigarro Muñoz, C. A., & Flórez Ramos, C. P. (2017). Estimación del área foliar en café variedad Castillo® a libre exposición y su relación con la producción. *Cenicafé*, 68(1), 55–61.
- Mukaka, M. (2012). Statistics corner: A guide to appropriate correlation coefficient in medical research. *Malawi Medical Journal*, 24(3), 69–71.
- Muñoz, J. D., Martínez, L. J., & Ligarreto, G. A. (2009). Caracterización de los ambientes agroecológicos del agraz o mortiño (*Vaccinium meridionale* Swartz), en la zona altoandina de Colombia. In G. A. Ligarreto (Ed.), *Perspectivas del cultivo de agraz o mortiño (Vaccinium meridionale Swartz) en la zona altoandina de Colombia* (pp. 29–56). Universidad Nacional de Colombia. Facultad de Agronomía.
- NeSmith, D. S. (1991). Nondestructive leaf area estimation of rabbiteye blueberries. *HortScience*, 26(10), Article 1332. <https://doi.org/10.21273/hortsci.26.10.1332>
- Nestby, R., Martinussen, I., Krogstad, T., & Uleberg, E. (2014). Effect of fertilization, tiller cutting and environment on plant growth and yield of European blueberry (*Vaccinium myrtillus* L.) in Norwegian forest fields. *Journal of Berry Research*, 4(2), 79–95. <https://doi.org/10.3233/JBR-140070>
- Panigrahi, N., & Sankar Das, B. (2020). Evaluation of regression algorithms for estimating leaf area index and canopy water content from water stressed rice canopy reflectance. *Information Processing in Agriculture*, 8(2), 284–298. <https://doi.org/10.1016/j.inpa.2020.06.002>
- PennState Eberly College of Science. (2021, January 20). *Lesson 13: Weighted Least Squares & Robust Regression*. <https://online.stat.psu.edu/stat501/lesson/13>
- Percival, D. C., & Privé, J. P. (2002). Nitrogen formulation influences plant nutrition and yield components of lowbush blueberry (*Vaccinium angustifolium* Ait.). *Acta Horticulturae*, 574, 347–353. <https://doi.org/10.17660/actahortic.2002.574.52>
- Percival, D. C., Janes, D. E., Stevens, D. E., & Sanderson, K. (2003). Impact of multiple fertilizer applications on plant growth, development, and yield of wild lowbush blueberry (*Vaccinium angustifolium* Aiton). *Acta Horticulturae*, 626, 423–429. <https://doi.org/10.17660/actahortic.2003.626.57>
- Pire, R., & Valenzuela, I. (1995). Estimación del área foliar en *Vitis vinifera* L. “French Colombard” a partir de mediciones lineales en hojas. *Agronomía Tropical*, 45(1), 143–154.
- Sánchez-Aguilar, H., Aldrete, A., Vargas-Hernández, J., & Ordaz-Chaparro, V. (2016). Influencia del tipo y color de envase en el desarrollo de plantas de pino en vivero. *Agrociencia*, 50(4), 481–492.
- Schloerke, B., Cook, D., Larmanange, J., Briatte, F., Marbach, M., Thoen, E., Elberg, A., Toomet, O., Crowley, J., Hofmann, H., & Wickham, H. (2021, June 21). GGally: Extension to ‘ggplot2’. Version 2.1.2. <https://CRAN.R-project.org/package=GGally>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- Signorell, A. (2018). DescTools: Tools for descriptive statistics. R package version 0.99.24. <https://cran.r-project.org/web/packages/DescTools/index.html>
- Singh, R. R., Meena, L. K., & Singh, P. (2017). High tech nursery management in horticultural crops: A way for enhancing income. *International Journal of Current Microbiology and Applied Sciences*, 6(6), 3162–3172. <https://doi.org/10.20546/ijcmas.2017.606.372>

- Shi, P., Ratkowsky, D. A., Li, Y., Zhang, L., Lin, S., & Gielis, J. (2018). A general leaf area geometric formula exists for plants - Evidence from the simplified Gielis equation. *Forests*, 9(11), Article 714. <https://doi.org/10.3390/f9110714>
- Spann, T. M., & Heerema, R. J. (2010). A simple method for non-destructive estimation of total shoot leaf area in tree fruit crops. *Scientia Horticulturae*, 125(3), 528–533. <https://doi.org/10.1016/j.scienta.2010.04.033>
- Suárez Salazar, J. C., Melgarejo, L. M., Duran Bautista, E. H., Di Rienzo, J. A., & Casanoves, F. (2018). Non-destructive estimation of the leaf weight and leaf area in cacao (*Theobroma cacao* L.). *Scientia Horticulturae*, 229, 19–24. <https://doi.org/10.1016/j.scienta.2017.10.034>
- Taiz, L., Zeiger, E., Moller, I. M., & Murphy, A. (2018). *Fundamentals of plant physiology*. Oxford University Press.
- Taugourdeau, S., le Maire, G., Avelino, J., Jones, J. R., Ramirez, L. G., Jara Quesada, M., Charbonnier, F., Gómez-Delgado, F., Harmand, J. M., Rapidel, B., Vaast, P., & Rouspard, O. (2014). Leaf area index as an indicator of ecosystem services and management practices: An application for coffee agroforestry. *Agriculture, Ecosystems and Environment*, 192, 19–37. <https://doi.org/10.1016/j.agee.2014.03.042>
- Torres, W. S., Montoya, I. A., & Ligarreto, G. A. (2009). Aspectos sociales y económicos de la producción de agraz o mortiño (*Vaccinium meridionale* Swartz). In G. A. Ligarreto, *Perspectivas del cultivo de agraz o mortiño (Vaccinium meridionale Swartz) en la zona altoandina de Colombia* (pp. 113–134).
- Torres, W. S., Rubio, E. W., & G. A. Ligarreto. (2012). Agraz o mortiño (*Vaccinium meridionale* Swartz). In G. Fischer (Ed.), *Manual para el cultivo de frutales en el trópico* (pp. 905–914). Produmedios.
- Wakui, A., & Kudo, G. (2021). Ecotypic differentiation of a circum-polar Arctic-alpine species at mid-latitudes: Variations in the ploidy level and reproductive system of *Vaccinium vitis-idaea*. *AoB PLANTS*, 13(3), Article plab015. <https://doi.org/10.1093/aobpla/plab015>
- Weraduwaage, S. M., Chen, J., Anozie, F. C., Morales, A., Weise, S. E., & Sharkey, T. D. (2015). The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science*, 6, Article 167. <https://doi.org/10.3389/fpls.2015.00167>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York. <https://ggplot2.tidyverse.org>
- Yan, G., Hu, R., Luo, J., Weiss, M., Jiang, H., Mu, X., Xie, D., & Zhang, W. (2019). Review of indirect optical measurements of leaf area index: Recent advances, challenges, and perspectives. *Agricultural and Forest Meteorology*, 265, 390–411. <https://doi.org/10.1016/j.agrformet.2018.11.033>
- Zapata-Vahos, I. C., Villacorta, V., Maldonado, M. E., Castro-Restrepo, D., & Rojano, B. (2015). Antioxidant and cytotoxic activity of black and green tea from *Vaccinium meridionale* Swartz leaves. *Journal of Medicinal Plants Research*, 9(13), 445–453. <https://doi.org/10.5897/JMPR2014.5744>