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BASIC AREAS
Article

Non-destructive models for estimating leaf area of guava cultivars

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ABSTRACT: Leaf area is a commonly used measurement in many agronomic studies, but its assessment is generally destructive, and then simple, accurate and non-destructive methods are really appreciated. The objective of this study was to develop a non-destructive model that could be used to estimate the leaf area of four guava (*Psidium guajava* L.) cultivars by using leaf linear dimensions. Leaves from guava cultivars 'Paluma', 'Sassaoka', 'Século 21', and 'Tailandesa' were sampled randomly from an experimental orchard. Leaf length and maximum leaf width were measured with a ruler in 120 leaves from each cultivar. Leaf areas were also measured with a leaf area meter. Linear and power models relating leaf area to length, width, and length × width were fitted to the data. The most precise models were regressed again with a new data set to validate the proposed models. The power model ($y = 0.61\,x^{1.06}$) using the length × width was more precise and accurate to estimate the leaf area of all four cultivars evaluated herein, grown in field or greenhouse conditions. When only one leaf dimension was used, the power model ($y = 1.81\,x^{1.93}$) using the width was the best-performing model. Although models with only one leaf dimension (length or width) have shown good performance for estimating the guava leaf area, models based on the leaf length × width were more precise.

Key words: *Psidium guajava*, guava leaf area, allometric method.

INTRODUCTION

Leaf area is an important variable used in physiological and agronomic studies, including light interception analysis, photosynthetic efficiency, evapotranspiration, and plant growth (Demirsoy 2009). In addition, leaf area may also be important in phytopathological studies to evaluate the effect of pathogens on host growth or even leaf damage (Amorim and Bergamin Filho 2018).

Leaf area meters are expensive, and sampling is usually destructive, which implies successive measurements of the same leaves are very difficult. Non-destructive leaf area measuring equipments do exist, but their cost is even higher than leaf area meters (Kumar 2009). Alternatives to leaf area meters include allometric methods, which are non-destructive and have high precision and simplicity (Rouphael et al. 2010).

In allometric methods, leaf area is estimated through mathematical models that incorporate linear measurements of leaf length and width (Blanco and Folegatti 2005). Such models have been developed for several fruit crops, such as cashew, fig, mango (Pandey and Singh 2011), persimmon (Cristofori et al. 2008), chestnut (Serdar and Demirsoy 2006), citrus (Mazzini et al. 2010), kiwi (Mendoza-de Gyves et al. 2007), medlar (Mendoza-de Gyves et al. 2008), papaya (Posse et al. 2009), peach (Demirsoy et al. 2004), strawberry (Demirsoy et al. 2005), grapevine (Montero et al. 2000; Williams III and Martinson 2003; Buttaro et al. 2015), muskmelon (Misle et al. 2013), and small fruit berries (Fallovo et al. 2008).

Although there are already two leaf area estimation models for guava, both were developed only with the 'Paluma' cultivar (Silva et al. 2015; Vitória et al. 2018). Then, the objective of this study was to develop a non-destructive model to estimate the leaf area of 'Paluma', 'Sassaoka', 'Século 21', and 'Tailandesa' guava cultivars.

MATERIAL AND METHODS

Four guava (*Psidium guajava*) cultivars, which are among those ones most planted in Brazil, were used to develop the leaf area models: 'Paluma', 'Sassaoka', 'Século 21', and 'Tailandesa' (Fig. 1). Leaves with varying size were sampled randomly from five trees of each cultivar grown in an experimental orchard (Piracicaba, SP, Brazil; 22°42'27"S, 47°37'42"W, altitude of 540 m a.s.l.). The guava trees were 5 years old, trained in vase shapes, and spaced at 3.5 × 3.5 m. Guava trees were pruned in August 2020, and samplings were carried out from February to July 2021.

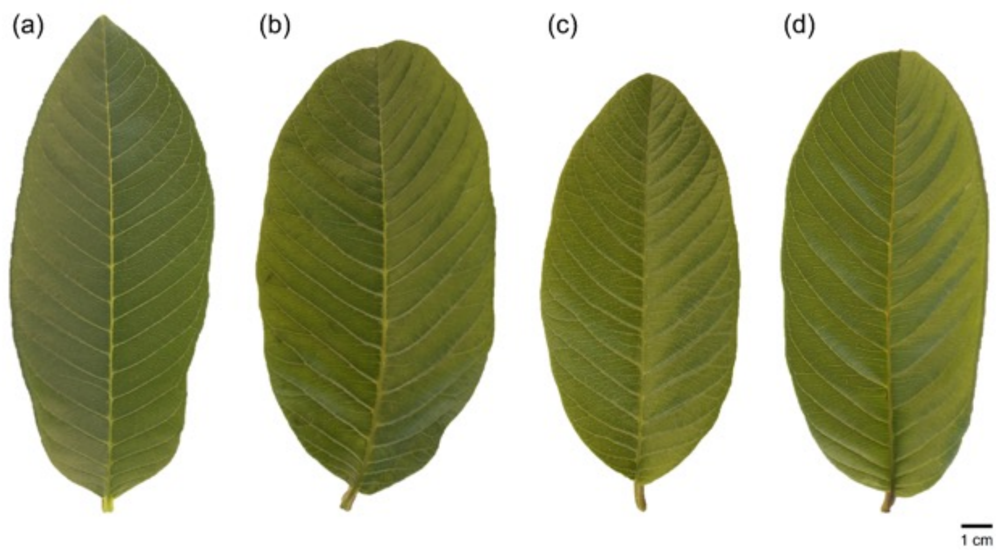

Figure 1. Leaves of (a) 'Paluma', (b) 'Sassaoka', (c) 'Século 21', and (d) 'Tailandesa' guava (*Psidium guajava*) cultivars.

Leaf length and width, and leaf area were measured on 120 leaves of each guava cultivar. Leaf length was defined as the distance from the petiole intersection to the leaf apex along the leaf blade. Both leaf length and maximum leaf width were measured with a ruler. The length-to-width ratio was calculated as the precision of leaf area estimates as it depends on the variation in leaf shape among cultivars (Rouphael et al. 2010). Finally, leaf area was measured with a leaf area meter model LI-3050 (LI-COR, Lincoln, NE, United States of America).

The utilization of both dimensions in modelling may introduce collinearity problems, which results in poor precision in the estimation of the regression coefficients. To detect the existence of collinearity, the variance inflation factor (VIF) and the tolerance values (T) were calculated (Marquardt 1970; Gill 1986). The VIF was estimated as Eq. 1:

$$VIF = 1/(1-R^2) \tag{1}$$

in which: R = the correlation coefficient between leaf length and width.

T was calculated as Eq. 2:

$$T = 1/VIF \tag{2}$$

VIF has to be lower than 10 and T greater than 0.10 to indicate that collinearity does not imply a real effect on the estimation through both dimensions. If collinearity does exist, then one of the leaf dimensions is excluded from the models.

Linear ($y = ax + b$) and power ($y = ax^b$) models were fitted to the data using the Statistica software (Version 7.0, StatSoft Inc., Tulsa, United States of America), considering the length (cm), width (cm), or length × width (cm^2) as the independent variables (x) and the measured leaf area (cm^2) as the dependent one (y). A linear model was fitted to the data in two ways: with and without the intersection (variable b in the equation) at 0. Regression with the intersection at 0 was performed to avoid estimates of negative values for leaf area. The choice of the best-performing model was based on the coefficient of determination (R^2), standard errors of estimates (SE), residual sum of squares (RSS), residual mean of squares (RMS), F-value, p-value, and dispersion pattern of the residuals (DPR).

Once the best fitted regression equations were selected, 240 leaves from cultivars 'Paluma', 'Sassaoka', 'Século 21', and 'Tailandesa' (60 leaves of each cultivar) grown in an experimental orchard were used to validate the proposed models and check their applicability to other guava cultivars. Additionally, the leaf area of 120 leaves of 'Paluma' guava plants grown in a greenhouse were measured. Leaf area of individual leaves was therefore estimated with each equation, and then regressed against the actual (measured) values using the Microsoft Office Excel software. For each model, the modified Willmott index *d* (Pereira et al. 2018) was calculated using R-software version 4.1.1 (R Core Team 2021). In addition, the mean error (ME), mean absolute error (MAE), and mean square error (MSE) were calculated using the Eqs. 3, 4, and 5:

$$ME = \frac{\sum_{i=1}^{n} \hat{Y}i - Yi}{n} \tag{3}$$

$$MAE = \frac{\sum_{i=1}^{n} \left|\hat{Y}i - Yi\right|}{n} \tag{4}$$

$$MSE = \frac{\sum_{i=1}^{n} \left(\hat{Y}i - Yi\right)^2}{n} \tag{5}$$

in which: $\hat{Y}i$ = estimated leaf areas; Yi = actual leaf areas; $\bar{Y}$ = the mean of actual leaf area; n = the total number of measured leaves.

The best combination here is the highest Willmott index and R^2, with the lowest estimation errors.

RESULTS AND DISCUSSION

The variance inflation factor (VIF) ranged from 2.08 and 5.56, and tolerance values (T) ranged from 0.18 and 0.48, depending on the cultivar. In all cultivars, VIF was lower than 10 and T greater than 0.10, indicating that the collinearity between length and width can be considered negligible and these two dimensions can be included in the model (Marquardt 1970; Gill 1986). The length-to-width ratios of leaves for all cultivars were close to 2 (Table 1). 'Paluma' and 'Sassaoka' presented the highest mean values of length, width, and leaf area. 'Paluma' also had highest maximum values for length, width, and leaf area, followed by 'Sassaoka', 'Tailandesa', and 'Século 21' (Table 1).

Length, width, and length × width were significantly ($p < 0.01$) correlated with the leaf area of all cultivars (Table 2 and Supplementary Table S1). Models based on the independent variables length and width separately had satisfactory correlation (Table 2 and Supplementary Table S1). The linear model showed a good fit when length or width were used alone as an independent variable, but with a non-normal dispersion pattern of the residuals in some cases (Table 2 and Supplementary Table S1). Furthermore, a problem of the linear model is the estimation of negative leaf area for small leaves (Table 2). The linear model with intersection at 0 did not show a good fit when length or width were used as an independent variable, and a non-normal dispersion pattern of the residuals was found (Table 2 and Supplementary Table S1). The power model showed the best fit to the guava leaf area of the four cultivars when only one leaf dimension (length or width) was used as an independent variable, especially when the four cultivars were pooled together (Table 2 and Supplementary Table S1, Fig. 2). These results were similar to those reported in other studies, in which the power model was the best when only one leaf dimension was used (Cargnelutti Filho et al. 2015; Silva et al. 2015; Pezzini et al. 2018). In fact, models with only one leaf dimension for leaf area estimation can provide good precision (Williams III and Martinson 2003; Rouphael et al. 2007; Kumar 2009; Pompelli et al. 2012), as shown herein (Table 2 and Supplementary Table S1, Fig. 2).

Table 1. Length-to-width ratio (L/W), minimum (min.), mean, and maximum (max.) values of length, width, and leaf area measured on 120 leaves of each guava (*Psidium guajava*) cultivar*.

Cultivar	L/W	Leaf length (cm)			Leaf width (cm)			Leaf area (cm^2)		
		Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
Paluma	1.99	3.00	12.26 (0.42)	19.00	1.70	6.26 (0.24)	10.80	4.20	72.77 (4.83)	183.32
Sassaoka	1.91	5.70	12.52 (0.24)	18.30	2.50	6.62 (0.13)	9.20	10.90	69.72 (2.30)	121.03
Século 21	2.01	3.50	10.04 (0.20)	14.50	2.20	4.99 (0.09)	6.80	6.23	42.97 (1.47)	78.74
Tailandesa	2.09	6.40	11.88 (0.19)	15.80	2.40	5.71 (0.09)	7.60	11.29	55.13 (1.65)	93.68

*Standard errors in parenthesis.

Table 2. Parameters and coefficients of determination (R^2) estimated by the fitted models that related the leaf areas of guava (*Psidium guajava*) cultivars to the independent variables length, width, and length × width*.

Model	Cultivar	*n**	Leaf length (L)			Leaf width (W)			Product (L × W)		
			a	*b*	R^2	*a*	*b*	R^2	*a*	*b*	R^2
Linear ($y = ax + b$)	Paluma	120	10.86	-60.41	0.90	20.04	-52.75	0.97	0.88	-5.19	0.99
	Sassaoka	120	9.36	-47.56	0.92	17.05	-43.19	0.91	0.80	0.72	0.98
	Século 21	120	6.86	-25.95	0.91	16.05	-37.18	0.90	0.82	0.19	0.97
	Tailandesa	120	8.25	-42.95	0.93	16.38	-38.36	0.88	0.78	0.57	0.97
	All cultivars	480	9.93	-55.75	0.88	18.79	-50.58	0.94	0.85	-2.80	0.99
Linear (intercept = 0) ($y = ax$)	Paluma	120	6.55	0	0.74	12.85	0	0.82	0.84	0	0.99
	Sassaoka	120	5.72	0	0.78	10.81	0	0.78	0.81	0	0.98
	Século 21	120	4.40	0	0.79	8.87	0	0.71	0.83	0	0.97
	Tailandesa	120	4.75	0	0.75	9.87	0	0.74	0.79	0	0.97
	All cultivars	480	5.50	0	0.69	10.92	0	0.76	0.82	0	0.98
Power ($y = ax^b$)	Paluma	120	0.09	2.57	0.98	1.65	1.98	0.99	0.45	1.13	0.99
	Sassaoka	120	0.77	1.77	0.92	1.91	1.88	0.93	0.88	0.98	0.98
	Século 21	120	0.70	1.77	0.93	1.63	2.01	0.91	0.85	0.99	0.97
	Tailandesa	120	0.58	1.83	0.93	2.37	1.79	0.89	0.85	0.98	0.97
	All cultivars	480	0.22	2.25	0.94	1.81	1.93	0.97	0.61	1.06	0.99

*Number of leaves. All regressions were significant ($p < 0.01$).

Leaf length × width was the independent variable that presented the highest coefficient of determination, and the lowest residual sum of squares and residual mean of squares for all fitted models and for all four guava cultivars (Table 2 and Supplementary Table S1, Fig. 2). Models based on length × width have been more accurate and been used for estimating the leaf area of several crops, such as hazelnut (Cristofori et al. 2007), coffee (Schmildt et al. 2015; Unigarro-Muñoz et al. 2015), soybean (Richter et al. 2014), millet (Leite et al. 2019), persimmon (Cristofori et al. 2008), citrus (Mazzini et al. 2010), sunflower (Aquino et al. 2011), kiwi (Mendoza-de Gyves et al. 2007), olive (Koubouris et al. 2018), grapevine (Montero et al. 2000), and small fruit berries (Fallovo et al. 2008). In most studies, the recommended models were linear and power models, which are simple and have high precision, thus corroborating our results.

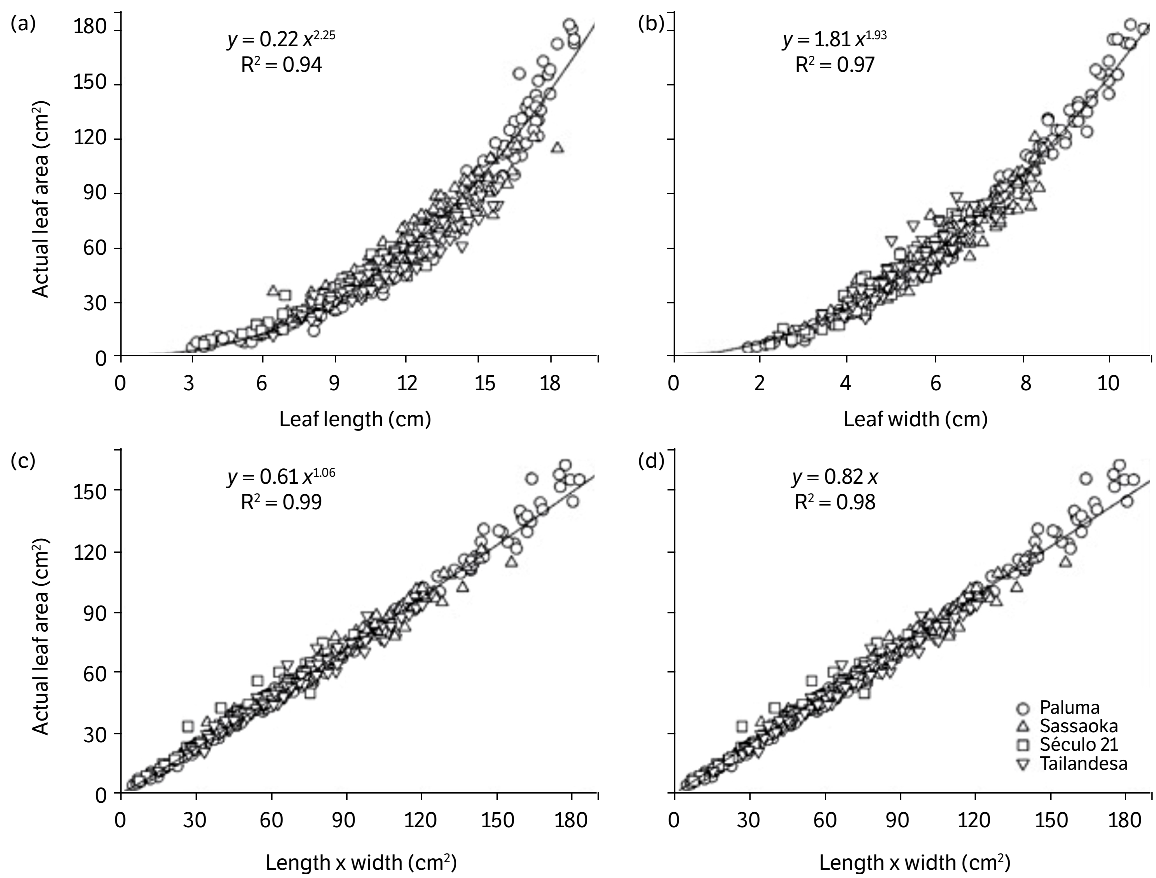

Figure 2. Relationships between (a) actual leaf area vs. leaf length, (b) width or (c and d) length × width for four guava cultivars pooled together and using (a-c) power or (d, intersection at 0) linear models ($n = 480$).

Overall, models validation revealed that the relationships between actual and estimated leaf areas have high coefficient of determination and Willmott index, as well as low values of mean error, mean absolute error, and mean square error (Table 3). Linear ($y = 0.82\ x$) and power ($y = 0.61\ x^{1.06}$) models, in which y is the leaf area and x is the leaf length × width, presented the highest coefficients of determination and Willmott index, and the lowest errors (Table 3 and Fig. 3). The relationships between actual and estimated leaf areas by the power model based on leaf length ($y = 0.22\ x^{2.25}$) and width ($y = 1.81\ x^{1.93}$) were satisfactory, but accuracy was reduced when compared to models based on length × width, especially when length was used as an independent variable (Table 3 and Fig. 3). Therefore, when only one leaf dimension was used, the power model is the most recommended, preferably using width as an independent variable.

Table 3. Parameters and statistical indices of regression between actual and estimated leaf area of guava (*Psidium guajava*). Coefficient of determination (R^2), Willmott index (d), mean error (ME), mean absolute error (MAE), and mean square error (MSE) for power model based on length (L), width (W) or length × width, and linear model with intersection at 0 based on length × width ($n = 360$ leaves).

Model	Equation	Independent variable	R^2	d	ME	MAE	MSE
Power	$y = 0.22\,x^{2.25}$	L	0.93	0.86	4.06	7.24	96.42
	$y = 1.81\,x^{1.93}$	W	0.95	0.89	-1.32	5.38	49.83
	$y = 0.61\,x^{1.06}$	L × W	0.99	0.96	-0.58	2.11	7.87
Linear	$y = 0.82\,x$	L × W	0.99	0.95	1.15	2.35	8.87

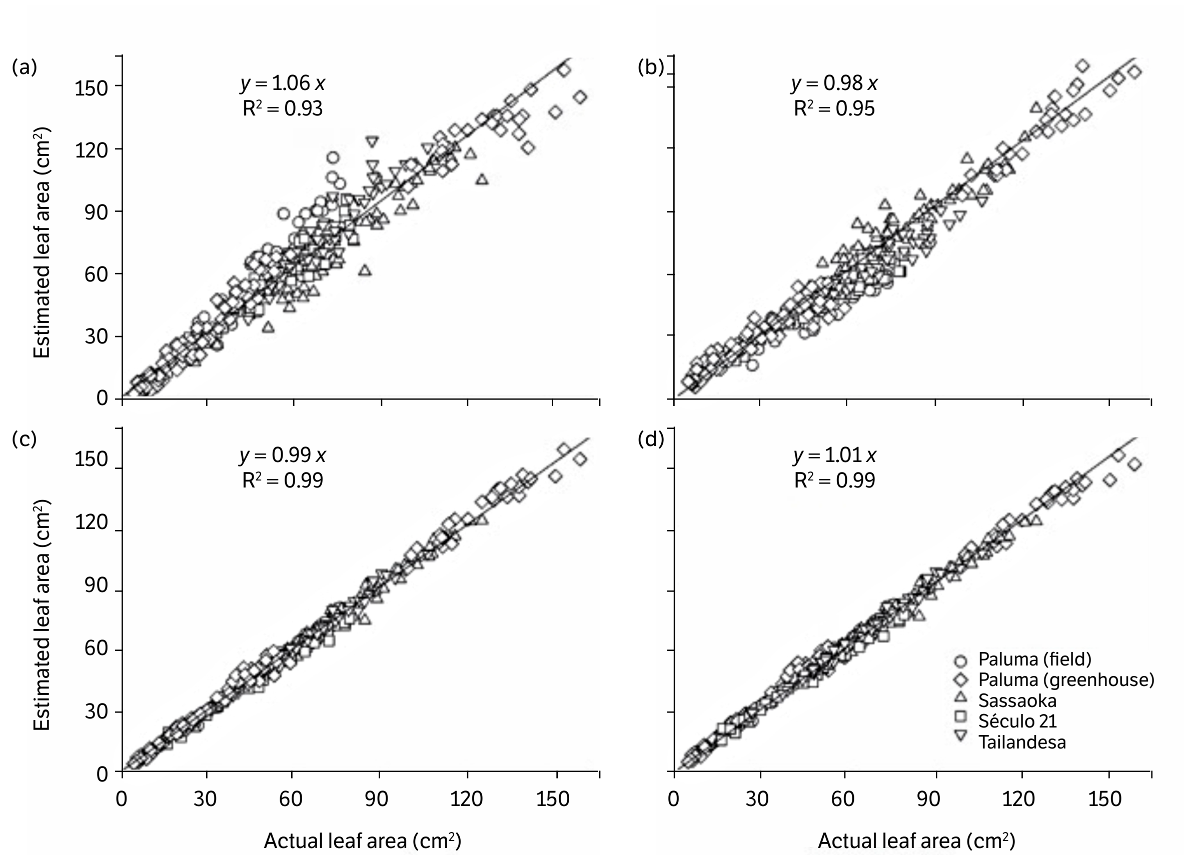

Figure 3. Estimation of leaf area for 'Paluma', 'Sassaoka', 'Século 21', and 'Tailandesa' guava cultivars pooled together (n = 360 leaves). Leaf area was estimated by using the power models with (a) leaf length ($y = 0.22\ x^{2.25}$), (b) leaf width ($y = 1.81\ x^{1.93}$), (c) leaf length × width ($y = 0.61\ x^{1.06}$), and a linear model (with intersection at 0) with (d) the leaf length × width ($y = 0.82\ x$). Lines represent the linear model with intersection at 0 ($y = ax$).

When length × width was used, both the power and linear (without intercept) models can be used (Table 3 and Fig. 3). Our results were in accordance to the models reported by Silva et al. (2015) for estimating the leaf area of 'Paluma' guava. Although models that use two leaf dimensions (length × width) have been more precise and accurate, models with only one leaf dimension (length or width) are less laborious and have been used to estimate accurately guava leaf area (Antunes et al. 2008; Santos et al. 2016; Pezzini et al. 2018), especially under field conditions.

The correlation between estimated and actual leaf area was very high (Fig. 3), and a single model, linear or power, is a simple way to estimate leaf area of all tested guava cultivars under different experimental conditions, such as field or greenhouse (Fig. 3). This is possible because the leaf shape (length-to-width ratios) of the guava cultivars was similar (Fig. 1). One must keep in mind that the accuracy of leaf area estimation depends on leaf shape, which may vary among cultivars. When cultivars have similar leaf shape, a single model is used to estimate leaf area (Cristofori et al. 2008; Mendoza-de Gyves et al. 2008; Rouphael et al. 2010). On the other hand, when there are differences in leaf morphology among cultivars, the use of a single model is an oversimplification and not recommended (Trachta et al. 2020). Non-destructive models are good alternatives to leaf area meters as these devices are expensive and most of them do not fit small guava leaves, causing leaf injuries and estimation errors.

CONCLUSION

The power model ($y = 0.61\ x^{1.06}$) using the product length × width was more appropriate to estimate the leaf area of 'Paluma', 'Sassaoka', 'Século 21', and 'Tailandesa' guava cultivars. When only one leaf dimension was used, the power model

($y = 1.81\ x^{1.93}$) using the width was the best-performing model, but accuracy was reduced when compared to models based on length × width.

AUTHORS' CONTRIBUTION

Conceptualization: Gonçalves, M. P., Ribeiro, R. V., and Amorim, L.; **Methodology:** Gonçalves, M. P., Ribeiro, R. V., and Amorim, L.; **Investigation:** Gonçalves, M. P.; **Data analysis:** Gonçalves, M. P.; **Writing – original draft:** Gonçalves, M. P.; **Writing – Review & Editing:** Gonçalves, M. P., Ribeiro, R. V., and Amorim, L.; **Funding Acquisition:** Amorim, L.; **Supervision:** Amorim, L. and Ribeiro, R. V..

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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SUPLEMENTARY MATERIAL

Table S1. Statistical indices estimated by the fitted models that relate the leaf area of guava (*Psidium guajava*) cultivars to the independent variables leaf length, width, and length × width. Standard errors of estimates (SE), residual sum of squares (RSS), residual mean of squares (RMS), F-value, *p*-value, and dispersion pattern of the residuals (DPR) are shown.

Model	Cultivars	Leaf length (L)							Leaf width (W)							Product (L × W)						
		SE		RSS	RMS	F-value	*p*-value	DPR*	SE		RSS	RMS	F-value	*p*-value	DPR*	SE		RSS	RMS	F-value	*p*-value	DPR*
		a	*b*						*a*	*b*						*a*	*b*					
Linear ($y = ax + b$)	Paluma	0.33	4.33	32,937.6	279.13	1,677.1	0.00	+	0.34	2.31	11,034.9	93.52	5,123.1	0.00	+	0.01	0.78	2,722.7	23.07	20,943.9	0.00	–
	Sassaoka	0.25	3.18	5,511.0	47.92	6,641.7	0.00	–	0.50	3.37	6,559.3	57.04	5,571.0	0.00	–	0.01	0.92	1,305.9	11.36	28,214.3	0.00	–
	Século 21	0.21	2.12	2,504.2	23.19	4,933.5	0.00	–	0.51	2.61	2,808.2	26.00	4,393.6	0.00	–	0.01	0.79	884.8	8.19	14,062.2	0.00	–
	Tailandesa	0.22	2.63	2,830.2	24.61	7,939.1	0.00	–	0.57	3.29	4,616.0	40.14	4,845.5	0.00	–	0.01	0.87	979.1	8.51	23,056.6	0.00	–
	All	0.17	2.07	64,620.9	139.87	7,762.7	0.00	+	0.23	1.41	34,567.7	74.82	14,712.4	0.00	+	0.00	0.40	7,454.5	16.14	69,063.6	0.00	–
Linear (intercept = 0) ($y = ax$)	Paluma	0.19		87,179.1	732.60	1,203.9	0.00	+	0.30		59,734.8	501.97	1,811.8	0.00	+	0.00		3,734.5	31.38	30,765.3	0.00	–
	Sassaoka	0.09		16,225.8	139.88	4,474.2	0.00	+	0.16		15,924.3	137.28	4,561.1	0.00	+	0.00		1,312.7	11.32	56,621.0	0.00	–
	Século 21	0.07		5,969.7	54.77	4,114.2	0.00	+	0.16		8,071.7	74.05	3,014.4	0.00	+	0.00		885.3	8.12	28,369.4	0.00	–
	Tailandesa	0.07		9,370.7	80.78	4,756.4	0.00	+	0.15		10,058.3	86.71	4,423.3	0.00	+	0.00		982.8	8.47	46,341.5	0.00	–
	All	0.07		165,976.6	358.48	5,774.9	0.00	+	0.13		130,628.6	282.14	7,462.9	0.00	+	0.00		8,265.4	17.85	124,801.2	0.00	–
Power ($y = ax^b$)	Paluma	0.01	0.06	7,623.4	64.61	7,442.1	0.00	–	0.10	0.03	3,489.1	29.57	16,330.4	0.00	–	0.03	0.01	1,863.9	15.80	30,620.4	0.00	–
	Sassaoka	0.12	0.06	5,737.1	49.89	6,377.6	0.00	–	0.25	0.06	5,198.8	45.21	7,043.9	0.00	–	0.06	0.02	1,296.4	11.27	28,420.9	0.00	–
	Século 21	0.10	0.06	2,058.6	19.06	6,013.1	0.00	–	0.20	0.07	2,477.8	22.94	4,986.8	0.00	–	0.07	0.02	884.5	8.19	14,067.0	0.00	–
	Tailandesa	0.08	0.05	2,840.8	24.70	7,909.2	0.00	–	0.30	0.07	4,366.4	37.97	5,125.8	0.00	–	0.06	0.02	974.3	8.47	23,170.9	0.00	–
	All	0.02	0.03	29,781.1	64.46	17,114.2	0.00	–	0.07	0.02	17,653.2	38.21	29,030.5	0.00	–	0.02	0.01	6,726.4	14.56	76,564.3	0.00	–

*Dispersion pattern of the residuals: – means normal or random dispersion; + means non-normal dispersion.