

Nondestructive estimation of leaf area in sour passion fruit seedlings using regression models

Estimativa não destrutiva da área foliar de mudas de maracujazeiro-azedo por modelos de regressão

João P. B. Souza¹ , Édlen dos S. Bonelá^{1*} , Leticia G. Morais¹ , Militino P. Carrafa¹ , Isaias dos S. Orgino¹ ,
Lucas A. de Souza¹ , Jalille A. Altoé¹ , Edilson R. Schimdt¹ 

¹Department of Agricultural and Biological Sciences, Universidade Federal do Espírito Santo, São Mateus, ES, Brazil.

ABSTRACT – Leaf area evaluation is essential for assessing plant photosynthetic efficiency, growth, dry matter accumulation, and crop yield potential. This study proposes equations to accurately estimate the leaf area of sour passion fruit seedlings ('BRS Rubi do Cerrado'). The experiment was conducted in a completely randomized design with four treatments, five replications, and 18 plants per plot, totaling 360 plants. Different substrate treatments were used to increase the variability of leaf dimensions and improve the robustness of the fitted equations. Treatments consisted of 100% commercial substrate (T1), 75% commercial substrate + 25% soil (T2), 50% commercial substrate + 50% soil (T3), and 25% commercial substrate + 75% soil (T4). Observed leaf area (OLA), length (LL), and width (LW) were evaluated 60 days after sowing in 1,561 leaves from plants in four replications to estimate leaf area equations as a function of LL, LW, and $LL \times LW$ using simple linear, power, and exponential models. LL^2 , LW^2 , and $(LL \times LW)^2$ were also used for the simple linear model. The equations were validated using data from the 340 leaves of the fifth replication. Observed leaf area was determined from digital images processed using ImageJ® software. Substrate type did not affect estimated leaf area (ELA) in passion fruit seedlings, which could be adequately obtained by the nondestructive method using the equations $ELA = 0.0913 + 0.7248 (LL \times LW)$ and $ELA = 0.7525 (LL \times LW)^{0.9906}$.

RESUMO – A avaliação da área foliar é essencial para estimar a eficiência fotossintética, o crescimento, o acúmulo de matéria seca e o potencial produtivo das plantas. Este estudo propõe equações para estimar com precisão a área foliar de mudas de maracujazeiro-azedo ('BRS Rubi do Cerrado'). O experimento foi conduzido em delineamento inteiramente casualizado, com quatro tratamentos, cinco repetições e 18 plantas por parcela, totalizando 360 plantas. Diferentes tratamentos com substrato foram utilizados para ampliar a variabilidade das dimensões foliares e aumentar a robustez das equações ajustadas. Os tratamentos consistiram em 100% substrato comercial (T1), 75% substrato comercial + 25% solo (T2), 50% substrato comercial + 50% solo (T3) e 25% substrato comercial + 75% solo (T4). A área foliar observada (AFO), o comprimento (C) e a largura (L) foram avaliados aos 60 dias após a semeadura em 1.561 folhas de plantas de quatro repetições, para estimar equações de área foliar em função de C, L e $C \times L$, utilizando modelos linear simples, potência e exponencial. C^2 , L^2 e $(C \times L)^2$ também foram utilizados no modelo linear simples. As equações foram validadas com dados das 340 folhas da quinta repetição. A área foliar observada foi determinada a partir de imagens digitais processadas no software ImageJ®. O tipo de substrato não afetou a área foliar estimada (AFE) em mudas de maracujazeiro, que pôde ser obtida adequadamente pelo método não destrutivo utilizando as equações $AFE = 0,0913 + 0,7248 (C \times L)$ e $AFE = 0,7525 (C \times L)^{0,9906}$.

Keywords: *Passiflora edulis* Sims f. *flavicarpa* O. Degener × *Passiflora edulis* Sims f. *edulis* 'BRS Rubi do Cerrado'. Mathematical models. Nondestructive methods. Equation fitting.

Palavras-chave: *Passiflora edulis* Sims f. *flavicarpa* O. Degener × *Passiflora edulis* Sims f. *edulis* 'BRS Rubi do Cerrado'. Modelos matemáticos. Métodos não destrutivos. Ajuste de equações.

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***Corresponding author:**
<edlen.bonela@live.com>

INTRODUCTION

Sour passion fruit (*Passiflora edulis* Sims) is a woody, perennial, vigorous climbing plant that produces rounded to oval fruits (THAKUR; TOPNO; JOSEPH, 2025). Native to tropical regions of the Americas, passion fruit is cultivated for its edible fruits and nutritional value, and passion fruit extracts have global commercial value in the pharmaceutical and personal care industries (DUARTE et al., 2021). Brazil leads global passion fruit production, with 736,585 Mg harvested from 47,082 ha in 2024, an average yield of 15,645 kg ha⁻¹, and Bahia as the leading producing state (IBGE, 2024).

In this context, high-quality seedlings are essential for establishing productive orchards because they increase plant survival and early development (SILVA et al., 2024). Therefore, the effects of different substrate proportions on passion fruit seedling formation and leaf area must be understood. Leaf area evaluation is essential for assessing plant photosynthetic efficiency, growth, and dry matter accumulation (BUSATO et al., 2009). Leaf area is also closely related to crop yield and directly linked to photosynthesis (ADJI et al., 2021).

Leaf area can be measured by direct destructive, direct nondestructive, or indirect nondestructive methods (RIBEIRO et al., 2022; SANTOS et al., 2026). In

direct destructive methods, all plant leaves are generally collected, preventing successive measurements of the same leaves (HERNÁNDEZ-FERNÁNDEZ; JARMA-OROZCO; POMPELLI, 2021). Although direct nondestructive leaf area measurement is simple, it generally requires expensive equipment (AMORIM et al., 2024), and larger leaves sometimes cannot be evaluated because of the limited reading area of portable equipment (SANTOS et al., 2023). Indirect nondestructive leaf area measurement allows rapid, successive evaluations of the same plant (HERNÁNDEZ-FERNÁNDEZ; JARMA-OROZCO; POMPELLI, 2021), is more convenient and less expensive (SANTOS et al., 2023), and uses regression equations based on leaf length and width measurements (SANTOS et al., 2026).

Thus, an approach commonly reported in the literature for modeling plant leaf area is regression equation fitting (SCHMILDT; OLIVEIRA; DOUSSEAU-ARANTES, 2023). This nondestructive method estimates leaf area from leaf dimensions accurately and rapidly, does not require sophisticated equipment, and enables leaf area measurement throughout the crop growth cycle (OLIVEIRA et al., 2019).

Leaf area modeling has been used in several species, including *Eugenia uniflora* L. (OLIVEIRA et al., 2019), *Prunus dulcis* (Mill.) D.A. Webb (MORAIS et al., 2023), *Stevia rebaudiana* (Bertoni) Bertoni (HERNÁNDEZ-FERNÁNDEZ; JARMA-OROZCO; POMPELLI, 2021), *Thespesia populnea* (L.) Sol. ex Corrêa (RIBEIRO et al., 2023), *Salvia hispanica* L. (GOERGEN et al., 2021),

Spondias tuberosa Arruda ex Koster (AMORIM et al., 2024), and *Selenicereus undatus* (Haw.) D.R. Hunt (SANTOS et al., 2026).

No modeling studies using unilobular leaves of sour passion fruit seedlings were found in the literature. Therefore, this study proposes equations to accurately estimate the leaf area of sour passion fruit seedlings (*Passiflora edulis* Sims f. *flavicarpa* O. Degener × *Passiflora edulis* Sims f. *edulis* 'BRS Rubi do Cerrado'). Leaves from seedlings produced in different substrates were evaluated to increase the variability of leaf dimensions and improve the robustness of the fitted equations.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the experimental farm of the University Center of Northern Espírito Santo (CEUNES), Federal University of Espírito Santo (UFES), in São Mateus, northern Espírito Santo State, Brazil (18°40'33"S and 39°51'44"W) (Figure 1). The regional climate is classified as Aw, according to the Köppen classification, characterized by a humid tropical climate with a dry winter and a hot, rainy summer (ALVARES et al., 2013).

A completely randomized design was used, with four treatments, five replications, and 18 seedlings per plot, totaling 360 seedlings. The treatments consisted of four soil and commercial substrate (Provaso®) proportions (Table 1).



Figure 1. Location of the greenhouse used to conduct the experiment. State of Espírito Santo, Brazil (A); São Mateus, Espírito Santo (B); University Center of Northern Espírito Santo (CEUNES), Federal University of Espírito Santo (UFES) (C); and greenhouse at CEUNES (D).

Table 1. Treatments obtained from the soil and commercial substrate proportions (w w⁻¹) used to produce 'BRS Rubi do Cerrado' passion fruit seedlings.

Treatments	Soil (%)	Commercial substrate (%)
T1	0	100
T2	25	75
T3	50	50
T4	75	25

The mixtures used for the treatments were prepared using soil collected from the experimental farm at a depth of 0–20 cm classified as an Argissolo (SANTOS et al., 2025) with a sandy loam texture. The mixture proportions (w w⁻¹) were determined by weighing and subsequent homogenization.

Chemical and physical properties of the mixtures used in the experiment were analyzed in the Agronomic Laboratory for Soil, Leaf, and Water Analysis of the Graduate Program in Tropical Agriculture (PPGAT) at CEUNES (Table 2).

Table 2. pH, calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), sodium (Na), aluminum (Al), potential acidity (H + Al), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), sulfur (S), sum of bases (SB), effective cation exchange capacity (CEC), CEC at pH 7.0 (CEC₇), base saturation (BS), and organic matter (OM) of the substrate mixtures used in the experiment.

Treatments	pH H ₂ O	Ca ----- cmolc dm ⁻³ -----	Mg ----- cmolc dm ⁻³ -----	K ----- mg dm ⁻³ -----	P ----- mg dm ⁻³ -----	Na ----- mg dm ⁻³ -----	Al ----- cmolc dm ⁻³ -----	H+ Al ----- cmolc dm ⁻³ -----
T1	7.6	3.1	2.5	1309	184.2	119	0	0
T2	7.5	2.4	2.6	1309	147.6	114	0	0
T3	7.4	2	2.9	1109	162.8	99	0	0
T4	6.9	0.9	2.1	715	1	61	0	0

Treatments	Fe ----- mg dm ⁻³ -----	Mn ----- mg dm ⁻³ -----	Zn ----- mg dm ⁻³ -----	Cu ----- mg dm ⁻³ -----	B ----- mg dm ⁻³ -----	S ----- mg dm ⁻³ -----	SB ----- cmolc dm ⁻³ -----	CEC ----- cmolc dm ⁻³ -----	CEC ₇ ----- cmolc dm ⁻³ -----	BS ----- % -----	OM ----- % -----
T1	221.9	49.9	26.5	1.9	0	0	9.5	9.5	9.5	100	31.96
T2	219.5	51.2	27.1	1.5	0	0	8.8	8.8	8.8	100	26.22
T3	236.8	41.2	20.4	1.7	0	0	8.2	8.2	8.2	100	17.43
T4	194.8	26.6	13.3	1.2	0	0	5.1	5.1	5.1	100	11.67

T1 = 100% commercial substrate; T2 = 75% commercial substrate + 25% soil; T3 = 50% commercial substrate + 50% soil; T4 = 25% commercial substrate + 75% soil. Ca, Mg, and Al contents were determined by extraction with 1 mol L⁻¹ KCl; K was extracted by the Mehlich-1 method; and potential acidity (H + Al) was determined by the 0.5 mol L⁻¹ calcium acetate method.

The sour passion fruit cultivar used, 'BRS Rubi do Cerrado' (*Passiflora edulis* Sims f. *flavicarpa* O. Degener × *Passiflora edulis* Sims f. *edulis*), is widely cultivated in northern Espírito Santo State. Seeds were obtained from the Brazilian Agricultural Research Corporation (Embrapa Cassava and Tropical Fruits; Cruz das Almas, Bahia, Brazil) and sown at a depth of 1 cm in trays containing 290-cm³ plastic tubes. The seedlings were kept in a greenhouse under microsprinkler irrigation, which was applied four times per day for 10 min each. At 60 days after sowing, all leaves were removed to enable leaf area modeling. At this time, all leaves

were unilobular and were divided into two groups: replications 1-4 were used to estimate equations for each substrate treatment, totaling 1,561 leaves, whereas replication 5, with 340 leaves, was reserved to validate the equations of each model.

Leaves of 'BRS Rubi do Cerrado' sour passion fruit were digitized in TIFF format using an HP Deskjet F4280® scanner at a resolution of 75 dpi. Observed leaf area (OLA), length (LL), and width (LW) were evaluated using ImageJ® software (Figure 2).

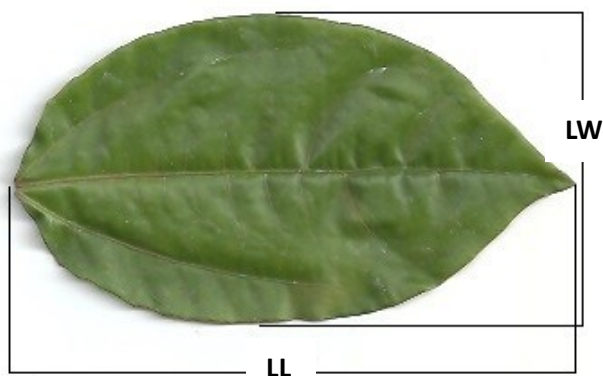


Figure 2. Shape of leaves from 'BRS Rubi do Cerrado' sour passion fruit seedlings used for leaf area modeling. LL = length measured along the leaf blade midrib; LW = greatest leaf blade width.

The following numbers of leaves were obtained for the four replications combined in each treatment: T1 = 421, T2 = 420, T3 = 385, and T4 = 335. Length, width, and observed leaf area (OLA) were compared among the four treatments using the Scott-Knott grouping test. Equations were estimated for leaf area as a function of length (LL), width (LW), and LL × LW using simple linear, power, and exponential models.

The data used were collected from simple unilobular leaves in each treatment and from the full set of 1,561 leaves across the four treatments. LL², LW², and (LL × LW)² were also used for the simple linear model, according to Cristofori et al. (2008). Sample representativeness was then evaluated by determining the minimum sample size (n_{eq}) at 3% using Equation 1 (SCHMILDT; OLIVEIRA; DOUSSEAU-ARANTES, 2023).

$$n_{eq} = \frac{t_{\alpha/2}^2 RMSE^2}{e^2 \overline{OLA}^2} \quad (1)$$

Where $t_{\alpha/2}^2$ is the Student's t value at 5% probability in the two-tailed table, e is the estimated error, in this case $e = 3\%$, $\overline{OLA}$ is the mean observed leaf area, and RMSE is the root mean square error, estimated using Equation 2:

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (ELA - \overline{OLA})^2}{n}} \quad (2)$$

Analysis of covariance (Ancova) was applied using Student's t-test at 5% probability to verify whether the same equation could be used for the different substrate treatments. In this analysis, the morphometric variable (LL, LW, or LL $\times$ LW) was the continuous covariate, and substrate was the factor used to test differences in intercept (β_0) and slope (β_1) among treatments. Linearization was performed before the analysis for the exponential and power models. The results were validated using root mean square error (RMSE) and Willmott's index of agreement (d), using Equation 3 (WILLMOTT, 1981):

$$d = 1 - \left[\frac{\sum_{i=1}^n (ELA - \overline{OLA})^2}{\sum_{i=1}^n (|ELA - \overline{OLA}| + |\overline{OLA} - \overline{OLA}|)^2} \right] \quad (3)$$

The equation used to estimate leaf area as a function of LL, LW, or LL $\times$ LW was selected based on the following analysis-of-covariance criteria: 1) if environment (substrate) affected β_0 and β_1 for all equations in all models, the best equation would be the one with, among the validation criteria, R^2 closest to one, RMSE closest to zero, Willmott's index of agreement closest to one, and ease of use according to Rouphael et al. (2007); 2) if environment did not affect β_0 and β_1 for at least one equation among the tested models (ZHANG; LIU, 2010), the equation should represent all study environments, in this case, substrate types, and have a simple model application (ROUPHAEL et al., 2007). The 1:1 line was plotted for the selected equation. Statistical analyses were performed using R software (R CORE TEAM, 2023) and the ExpDes.pt package (FERREIRA; CAVALCANTI; NOGUEIRA, 2022).

RESULTS AND DISCUSSION

The summary of the analysis of variance and mean comparison test (Table 3) revealed significant differences in observed leaf area (OLA), length (LL), and width (LW). Lower mean values were observed in leaves from plants grown in the mixture with the lowest commercial substrate content (T4). This pattern was also reported for *Jatropha curcas* L. seedlings (LIMA et al., 2009). Despite differences among treatments in OLA, LL, and LW, the existence of distinct leaf area fitting equations for the different treatments cannot be automatically inferred. Thus, modeling is needed to evaluate the dependence of OLA on LL, LW, and LL $\times$ LW.

Table 3. Summary of the analysis of variance and mean comparison test for leaf length (cm), leaf width (cm), and observed leaf area (cm²) of 'BRS Rubi do Cerrado' sour passion fruit seedlings 60 days after sowing in different substrates.

Source of Variation	Degrees of freedom	Mean square		
		Leaf length	Leaf width	Leaf area
Treatment	03	218.94**	75.14**	10468.42**
Residue	1557	5.63	1.63	207.08
CV	-	31.06	28.12	52.58
Minimum	-	1.37	0.61	1.19
Maximum	-	14.07	7.96	77.29
Mean ⁽¹⁾	T1	8.15a	4.80a	31.01a
	T2	8.27a	4.91a	31.80a
	T3	7.55b	4.53b	26.55b
	T4	6.55c	3.88c	19.60c

Significant at 1% by the F-test. Means followed by the same letter within each variable do not differ by the Scott-Knott grouping test at 5% probability. T1 = 100% commercial substrate; T2 = 75% commercial substrate + 25% soil; T3 = 50% commercial substrate + 50% soil; T4 = 25% commercial substrate + 75% soil.

In leaf area modeling studies, the sample should include a wide range of leaf dimensions because this increases model applicability to different leaf sizes. This indicates that the data are representative, with greater leaf-size variability within the sample and greater robustness in the analysis. Under these conditions, final models can be applied to a wide range of leaf dimensions (SANTOS et al., 2023). The best estimated leaf area (ELA) representation was obtained with

the simple linear models $ELA = \hat{\beta}_0 + \hat{\beta}_1 (LL \times LW)$ and $ELA = \hat{\beta}_0 + \hat{\beta}_1 (LL \times LW)^2$ and the power model $ELA = \hat{\beta}_0 (LL \times LW)^{\hat{\beta}_1}$.

Although significant differences in LL, LW, and OLA were observed among substrate treatments (Table 3), analysis of covariance (Ancova) indicated that a single equation could be fitted for seedling leaves across these substrates. This result is explained by the fact that, in modeling, the effect of the

environment on LL, LW, and OLA is less important than its effect on the allometric relationship between OLA and the leaf dimensions used in the different models (SANTOS et al., 2023). This pattern was reported in leaf area modeling studies of *Berberia purpurascens* grown at different altitudes in Tibet (ZHANG; LIU, 2010), wild passion fruit (*Passiflora mucronata*) grown in the field and in a greenhouse (SCHMILDT et al., 2016), and cherimoya (*Annona cherimola*) grown in full sunlight and under protected cultivation (SCHMILDT et al., 2017).

Ancova results are shown for four cases in Figure 3. Ancova was significant when LL was used as the explanatory

variable for leaf area in the simple linear model (Figure 3A; Table 4). Although the fitted lines have the same direction, the intercepts ($\hat{\beta}_0$) and the slopes ($\hat{\beta}_1$) differ among treatments. The same pattern was observed when leaf width (LW) was used as the explanatory variable in the simple linear model (Figure 3B). These differences were not observed when $LL \times LW$ was used as the explanatory variable for leaf area in the simple linear model (Figure 3C) or in the power model (Figure 3D), which supports the nonsignificant Ancova results (Table 4) observed for the simple linear and power models using $LL \times LW$ as the explanatory variable.

Table 4. Intercepts ($\hat{\beta}_0$), slopes ($\hat{\beta}_1$), analysis of covariance (Ancova), coefficients of determination (R^2), and minimum sample sizes required to obtain the equations (n_{eq}) for estimated leaf area (ELA) models of 'BRS Rubi do Cerrado' sour passion fruit based on leaf length (LL) and leaf width (LW) of seedlings grown in different substrates.

Model	Treatment	$\hat{\beta}_0$	$\hat{\beta}_1$	Ancova		R^2	n_{eq}
				$\hat{\beta}_0$	$\hat{\beta}_1$		
$ELA = \hat{\beta}_0 + \hat{\beta}_1 LL$	T1	-17.8557	5.9903			0.9358	85
	T2	-19.3954	6.1735			0.9354	72
	T3	-16.5280	5.7304			0.9282	71
	T4	-13.1168	5.0129			0.9382	70
	Set	-17.8825	5.9268	*	*	0.9347	85
$ELA = \hat{\beta}_0 + \hat{\beta}_1 LW$	T1	-22.7221	11.1767			0.9392	80
	T2	-23.8957	11.3593			0.9337	74
	T3	-21.0856	10.5437			0.9267	72
	T4	-17.6360	9.6307			0.9276	81
	Set	-22.3264	10.9635	*	*	0.9358	83
$ELA = \hat{\beta}_0 + \hat{\beta}_1 (LL \times LW)$	T1	0.2041	0.7194			0.9966	5
	T2	-0.0654	0.7310			0.9966	4
	T3	0.0447	0.7265			0.9959	4
	T4	0.2030	0.7198			0.9959	5
	Set	0.0913	0.7248	ns	ns	0.9966	5
$ELA = \hat{\beta}_0 + \hat{\beta}_1 LL^2$	T1	1.4236	0.9520			0.9531	61
	T2	1.4078	0.9657			0.9531	57
	T3	1.5539	0.9601			0.9531	59
	T4	1.2007	0.9289			0.9531	51
	Set	1.2126	0.9617	ns	ns	0.9531	61
$ELA = \hat{\beta}_0 + \hat{\beta}_1 LW^2$	T1	1.6137	0.9590			0.9501	54
	T2	1.5368	0.9633			0.9501	54
	T3	1.5316	0.9475			0.9501	52
	T4	1.2121	0.9783			0.9501	57
	Set	1.4870	0.9603	ns	ns	0.9501	56

T1 = 100% commercial substrate; T2 = 75% commercial substrate + 25% soil; T3 = 50% commercial substrate + 50% soil; T4 = 25% commercial substrate + 75% soil. For Ancova, * and ns indicate significant and nonsignificant effects, respectively, at 5% by the t-test in comparisons among treatments.

Table 4. Continuation.

Model	Treatment	$\hat{\beta}_0$	$\hat{\beta}_1$	Ancova		R^2	n_{eq}
				$\hat{\beta}_0$	$\hat{\beta}_1$		
$ELA = \hat{\beta}_0 + \hat{\beta}_1(LL + LW)^2$	T1	0.3957	0.9907			0.9937	10
	T2	0.1432	1.0077			0.9937	9
	T3	0.2529	1.0033			0.9937	9
	T4	0.4287	0.9848			0.9937	9
	Set	0.2708	0.9993	ns	ns	0.9937	10
$ELA = \hat{\beta}_0 LL^{\hat{\beta}_1}$	T1	0.6240	1.8206			0.9549	60
	T2	0.6040	1.8400			0.9498	56
	T3	0.6221	1.8263			0.9408	59
	T4	0.6493	1.7824			0.9550	51
	Set	0.6120	1.8293	*	*	0.9537	60
$ELA = \hat{\beta}_0 LW^{\hat{\beta}_1}$	T1	1.2447	1.9857			0.9595	54
	T2	1.2248	1.9979			0.9514	55
	T3	1.2516	1.9741			0.9470	53
	T4	1.1723	2.0277			0.9490	58
	Set	1.2256	1.9940	*	*	0.9571	57
$ELA = \hat{\beta}_0(LL \times LW)^{\hat{\beta}_1}$	T1	0.7821	0.9795			0.9965	5
	T2	0.7333	0.9984			0.9966	4
	T3	0.7428	0.9943			0.9958	4
	T4	0.7454	0.9928			0.9959	5
	Set	0.7525	0.9906	ns	ns	0.9966	5
$ELA = \hat{\beta}_0 e^{\hat{\beta}_1 LL}$	T1	2.9446	0.2630			0.9137	174
	T2	3.2196	0.2559			0.9087	139
	T3	2.9761	0.2692			0.9142	108
	T4	2.5401	0.2905			0.9219	123
	Set	2.9757	0.2654	*	*	0.9139	164
$ELA = \hat{\beta}_0 e^{\hat{\beta}_1 LW}$	T1	2.3596	0.4923			0.8988	230
	T2	2.6383	0.4734			0.9036	154
	T3	2.3487	0.5002			0.9025	136
	T4	1.9061	0.5647			0.9116	139
	Set	2.4157	0.4931	*	*	0.9038	199
$ELA = \hat{\beta}_0 e^{\hat{\beta}_1(LL \times LW)}$	T1	7.0381	0.0297			0.8662	421
	T2	7.6026	0.0289			0.8739	298
	T3	6.8746	0.0326			0.8966	202
	T4	5.7678	0.0399			0.8790	298
	Set	7.1093	0.0307	*	*	0.8593	426

T1 = 100% commercial substrate; T2 = 75% commercial substrate + 25% soil; T3 = 50% commercial substrate + 50% soil; T4 = 25% commercial substrate + 75% soil. For Ancova, * and ns indicate significant and nonsignificant effects, respectively, at 5% by the t-test in comparisons among treatments.

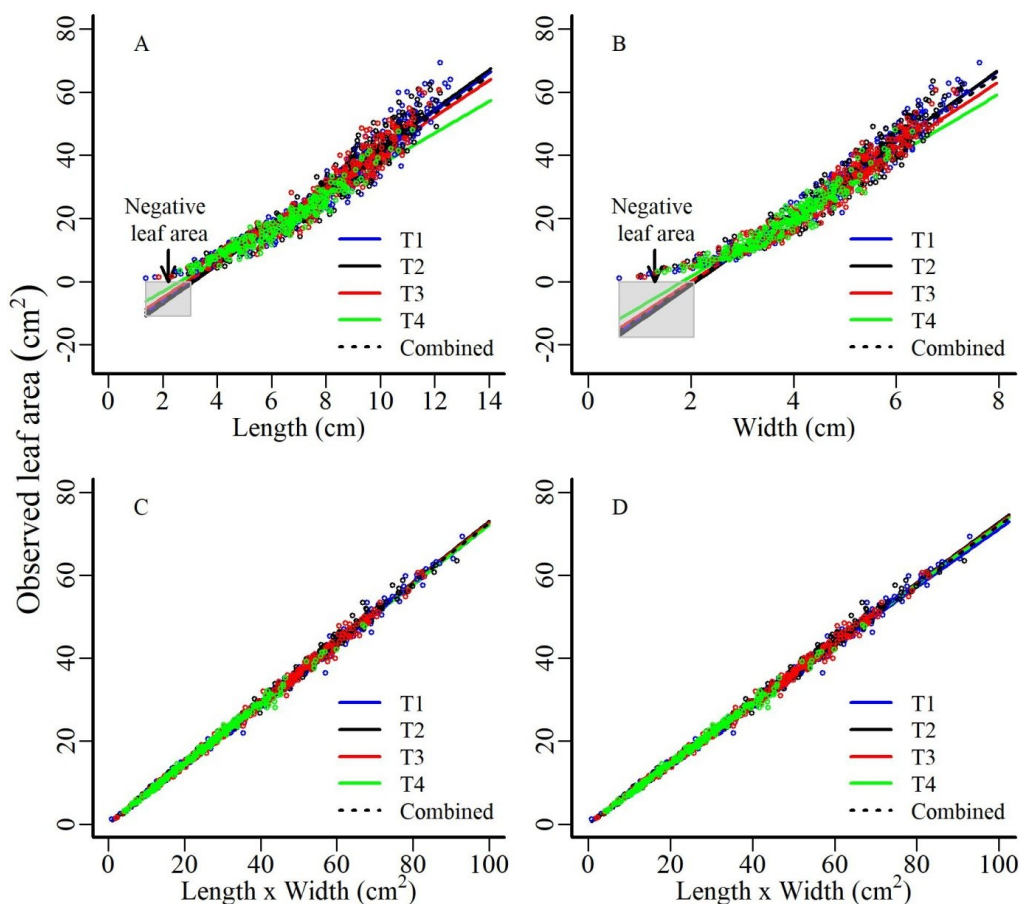


Figure 3. Fitted lines representing leaf area of ‘BRS Rubi do Cerrado’ sour passion fruit seedlings as a function of leaf blade dimensions 60 days after sowing in different substrates and for the full set of leaves. Leaf length in the simple linear model (A); leaf width in the simple linear model (B); leaf length $\times$ width in the simple linear model (C); leaf length $\times$ width in the power model (D). T1 = 100% commercial substrate; T2 = 75% commercial substrate + 25% soil; T3 = 50% commercial substrate + 50% soil; T4 = 25% commercial substrate + 75% soil.

The use of LL as the only independent variable in the simple linear model may result in negative ELA values for smaller leaves. For the joint equation $ELA = -17.8825 + 5.9268LL$, ELA is negative when $LL \leq 3.02$ cm. Negative values would occur in this case (Figure 3A) because the lowest observed LL value was 1.37 cm (Table 3). Thus, the simple linear model using only LL as the independent variable is not recommended. The same limitation occurs when LW is used in the simple linear model. For the joint equation $ELA = -22.3264 + 10.9635LW$, ELA is negative when $LW \leq 2.04$ cm. Negative estimated leaf area values would occur (Figure 3B) because the lowest observed LW value was 0.61 cm (Table 3). Bonelá et al. (2022) stated that models resulting in negative values should not be selected because they do not reflect biological reality.

Considerable variation was observed in the minimum sample sizes required to obtain the equations (n_{eq}), with greater variation among the tested models than among the measurements used as independent variables (Table 4). The analysis of leaves from different substrates showed a higher n_{eq} for the exponential model when $LL \times LW$ was used as the independent variable, indicating that the exponential model was the least adequate for fitting leaf area in passion fruit

seedlings.

This pattern was also observed by Schmildt, Oliveira and Dousseau-Arantes (2023) in leaf area modeling of cattley guava (*Psidium cattleianum*) and by Morais et al. (2023) in leaf area modeling of almond (*Prunus dulcis*). Thus, at least $n_{eq} = 426$ leaves of different sizes are required for leaf area modeling studies of passion fruit seedlings. In this study, 1,561 leaves were used, exceeding the required n_{eq} and supporting the reliability of the equations estimated in the modeling study (Table 4).

Table 5 presents the validation statistics obtained from 340 leaves, relating observed leaf area (OLA) to estimated leaf area (ELA) for the 12 equations estimated from the full leaf dataset. In the linear regression between OLA and ELA, both coefficients, $\hat{\beta}_0$ and $\hat{\beta}_1$, were nonsignificant for the equations that used $LL \times LW$ as the explanatory variable. Among these equations, the simple linear equation $ELA = 0.0913 + 0.7248(LL \times LW)$ and the power equation $ELA = 0.7525(LL \times LW)^{0.9906}$ were the most adequate for estimating leaf area in ‘BRS Rubi do Cerrado’ sour passion fruit seedlings. These equations had the most adequate R^2 , RMSE, and d values in the validation analysis (Table 5).

Table 5. Fitted models with their respective independent variables (x), intercepts ($\hat{\beta}_0$), slopes ($\hat{\beta}_1$), coefficients of determination (R^2), root mean square error (RMSE), and Willmott's index of agreement (d; WILLMOTT, 1981), obtained from regressions between estimated leaf area (ELA) and observed leaf area (OLA) for the models tested in a sample of 340 leaves from 'BRS Rubi do Cerrado' sour passion fruit seedlings.

Fitted models	x	$\hat{\beta}_0^{(1)}$	$\hat{\beta}_1^{(2)}$	R^2	RMSE	d
Linear	LL	3.0818**	0.8966**	0.9302	4.1392	0.9806
Linear	LW	3.7242**	0.8844**	0.9357	4.0395	0.9813
Linear	LL × LW	0.2090 ^{ns}	1.0024 ^{ns}	0.9972	0.8699	0.9992
Linear	LL ²	1.2126 ^{ns}	0.9617**	0.9531	3.3680	0.9879
Linear	LW ²	1.4870 ^{ns}	0.9603**	0.9501	3.4905	0.9870
Linear	(LL + LW) ²	0.2708 ^{ns}	0.9993 ^{ns}	0.9937	1.2611	0.9984
Power	LL	1.2918 ^{ns}	0.9530**	0.9532	3.3608	0.9879
Power	LW	1.5472 ^{ns}	0.9474**	0.9501	3.4681	0.9870
Power	LL × LW	0.3593 ^{ns}	0.9955 ^{ns}	0.9972	0.8556	0.9992
Exponential	LL	-4.5941**	1.1878**	0.9038	6.7374	0.9619
Exponential	LW	-5.3139**	1.2268**	0.8908	7.6434	0.9530
Exponential	LL × LW	-11.4103**	-10.4638**	0.8517	12.0817	0.9064

** = intercept differs from zero, or slope differs from one, by the t-test at a 1% probability level of error; ns = not significant at 5%.

In many leaf area modeling studies, the linear model using LL × LW provides the best fit, as reported by Morgado et al. (2013) for leaf area in *Passiflora* species and by Amorim et al. (2024) for *Spondias tuberosa* Arruda ex Koster. The best-fit equations recommended for the simple linear and

power models, as well as their validation using the 1:1 line, are shown in Figure 4 and indicate an excellent fit. The equations $ELA_i = 0.0913 + 0.7248 (LL \times LW)_i$ and $ELA_i = 0.7525 (LL \times LW)_i^{0.9906}$ can be used for all substrate composition treatments.

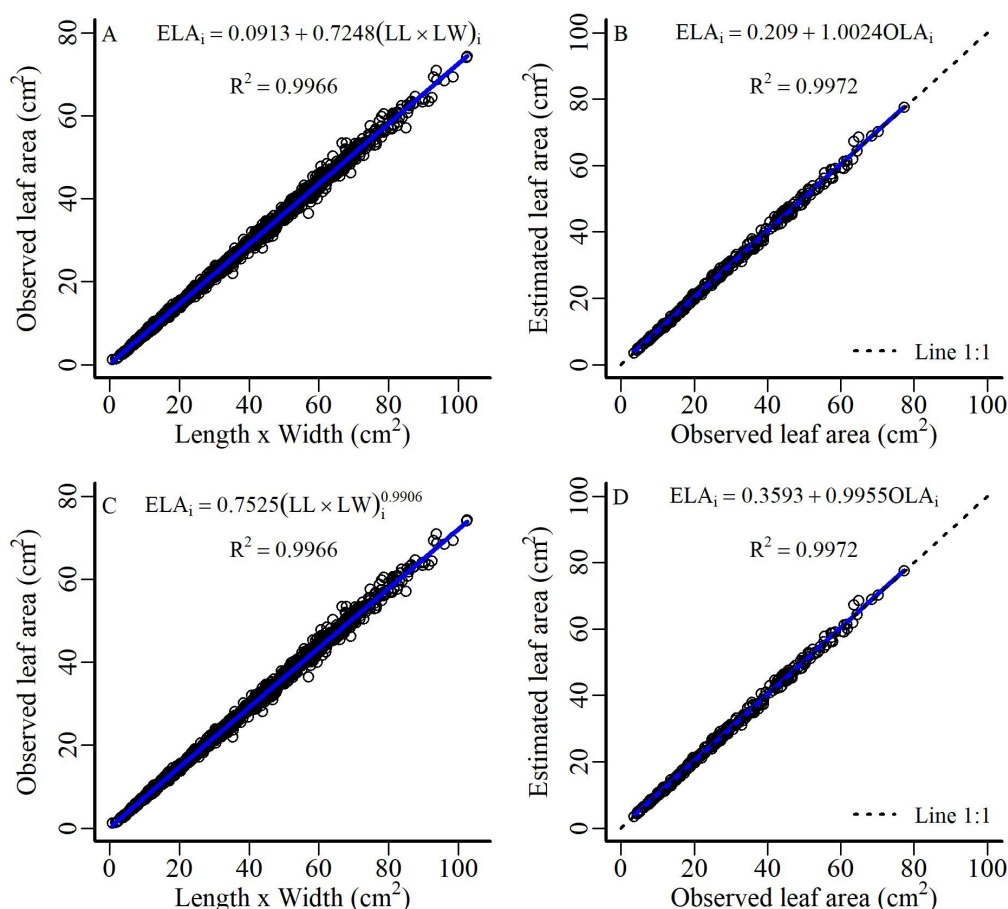


Figure 4. Simple linear function modeled from leaves of 'BRS Rubi do Cerrado' sour passion fruit seedlings grown in different substrates (n = 1,561 leaves) (A); 1:1 line for the relationship between estimated leaf area and observed leaf area in the selected simple linear model (n = 340 leaves) (B); power function modeled from leaves of passion fruit seedlings grown in different substrates (C); 1:1 line for the relationship between estimated leaf area and observed leaf area in the selected power model (D).

CONCLUSION

Although the substrates affected seedling dimensions and observed leaf area, they did not alter the allometric relationship between leaf area and the length $\times$ width product. Thus, the leaf area of 'BRS Rubi do Cerrado' sour passion fruit seedlings can be estimated by a nondestructive method from LL and LW, preferably by the equations $ELA = 0.0913 + 0.7248 (LL \times LW)$ and $ELA = 0.7525 (LL \times LW)^{0.9906}$.

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