

# Non-destructive model to estimate the leaf area of multiple Vochysiaceae species

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**Abstract** Leaf area (LA) is commonly used to evaluate plant traits such as leaf development and plant growth, which depend on sunlight interception. However, methods for obtaining LA are destructive and require many leaf samples that might not be available. The use of linear equations to estimate LA without destructive procedures has been successfully used in crops and weeds studied in the field and in potted-plant experiments. In the Brazilian savanna (Cerrado), many studies still depend on destructive leaf sampling because equations would be specific for each species. We developed a non-destructive model to estimate LA of five Vochysiaceae species from the cerrado, as this family exclusively comprised AI-accumulating species. In this study, LA versus leaf length (L) and leaf width (W) was regressed, and the best-fitted model ( $LA = -2.133 + 0.719 LW$ ,  $R^2 = 0.99$ ) was validated to estimate LA in *Qualea cordata* Spreng., *Qualea grandiflora* Mart., *Salvertia convallariodora* A. St.-Hil., *Vochysia cinnamomea* Pohl., and *Vochysia tucanorum* Mart. with precise accuracy. This model may help scientists with distinct interests in plants from this family.

**Keywords** Brazilian savanna · Cerrado · Linear measurements · Mathematical models

## Introduction

Leaf area (LA) is largely explored in studies of leaf development, plant growth, and sunlight interception (Blanco and Folegatti 2005; Lusk et al. 2008). However, most of the techniques used to estimate LA require excising leaves from plants and scanning them in the lab. When considering different treatments, big populations, or several samples, destructive procedures can be time-consuming. These techniques may also interfere on physiological and phenological responses due to canopy reduction (Chabot and Hicks 1982). On the other hand, there are portable LA meters available on the market, such as the LI-3000C portable meter (LI-COR, Lincoln, NE, USA). However, these instruments may cost some thousands of dollars.

Equations derived from non-destructive models, when available, represent a free and fast method that can be used in situ, providing LA estimates without leaf excisions (Norman and Campbell 1989). One of the most frequently used non-destructive models to estimate LA is based on equations using leaf length (L) and width (W) (Blanco and Folegatti 2005). These measurements can be easily made in the field and greenhouse using a ruler.

These non-destructive models are generally linear equations, making it very easy to the user to obtain the final LA using a simple calculator. Therefore, these models have long been used to estimate LA in crops [e.g., *Zingiber officinale* Roscoe (Ancy and Jayachandran 1994), *Vitis labruscana* L.H. Bailey (Williams and Martinson 2003), *Cucumis sativus* L., *Cucurbita* spp. (Blanco and Folegatti 2005), *Vicia faba* L. (Peksen 2007), *Rosa hybrida* E.H.L.Krause,

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(Rouphael et al. 2010), *Calendula officinalis* L., *Dahlia pinnata* Cav., *Dianthus barbatus* L., and *Viola wittrockiana* Gams ex Nauenb. & Buttler (Giuffrida et al. 2011)] and weeds [*Amaranthus* spp. (Carvalho and Christoffoleti 2007), *Ageratum conyzoides* (L.) L. (Bianco et al. 2008), *Pistia stratiotes* L. (Carvalho et al. 2011a), *Merremia cissoides* (Lam.) Hallier f. (Carvalho et al. 2011b)]. The massive usage of these models for studying crops and weeds may have two interpretations. These species might require studies in which LA is extremely important, especially because plant growth analysis is paramount in the interaction between crops and weeds. Another interpretation is that LA is difficult to assess, especially in tree species, and that is why studies with crops and weeds benefit from these models.

The Brazilian savanna (cerrado), for instance, has been recognized as a global biodiversity hotspot with more than 10,000 plant species, of which 44 % are endemic (Simon et al. 2009). However, whole-plant studies, trying to understand LA enlargement or its oscillation over time, are not so frequent in studies involving forests and savannas. As a result, few studies of cerrado woody species include LA measurements. Just recently, Souza and Habermann (2014) developed the first non-destructive model to estimate LA in *Styrax pohlii* A. DC., and *Styrax ferrugineus* Nees & Mart. (Styracaceae), congeners from Cerrado areas, and these models were used in a study with *S. pohlii* potted plants (Kissmann et al. 2014). Similar models were proposed to measure LA in *Vernonia ferruginea* Less. (Souza and Amaral 2015), another cerrado woody species.

Due to difficulty in measuring LA in tree species, specific leaf area (SLA, ratio of the leaf area per leaf dry mass) is used as an alternative trait to estimate how LA could vary in response to several conditions. In cerrado vegetation, an important condition is certainly the high aluminum (Al) concentration in the soil (Haridasan 2008). As a result, Al-accumulating and non-accumulating species coexist, growing on acidic (pH < 4.0) soils with low fertility. In this scenario, the increase in SLA is slower in Al-accumulating plants as compared to non-accumulating species (Souza et al. 2015), suggesting that in the cerrado, Al accumulation is associated with LA changes, although LA was never directly studied under such perspective due to a lack of non-destructive LA models for field studies.

The Vochysiaceae family comprises eight genera, and approximately 200 species of trees and shrubs distributed throughout tropical regions of the Americas and Africa (Carnevale Neto et al. 2011). In Brazil, *Qualea* and *Vochysia* are the most common genera of Vochysiaceae in the Cerrado domain, and this family exclusively comprised Al-accumulating species (Haridasan 1982; Medeiros and Haridasan 1985; Haridasan and Araújo 1988; Souza et al. 2015). In addition, non-destructive models to measure LA are not available for this important Al-accumulating

family. Due to the importance of this family in the cerrado (Sajo and Rudall 2002; Haridasan 2008), these plants are not only studied under the Al accumulation perspective, but also from ecological, botanical, and functional standpoints (Paulilo et al. 1993; Barbosa et al. 1999; Haridasan 2008). Therefore, we developed and validated a linear model to predict LA of five Vochysiaceae species (*Qualea cordata* Spreng., *Q. grandiflora* Mart., *Salvertia convallariodora* A. St-Hil., *V. cinnamomea* Pohl., and *V. tucanorum* Mart.).

## Materials and methods

### Plant material and site description

Leaves of *Qualea cordata*, *Q. grandiflora*, *Salvertia convallariodora*, *Vochysia cinnamomea*, and *V. tucanorum* were collected from adult trees (2–3 m in height) growing at the Bauru Botanic Garden (22°20'39"S e 49°00'51"W) in September 2014. Bauru is a city located in the center of São Paulo state, southeastern Brazil.

The vegetation of Bauru Botanic Garden (322 ha) comprised 'cerradão' and cerrado sensu stricto, which are forest- and savanna-type physiognomies, respectively, typical of Cerrado areas; semideciduous and gallery forests are also part of the landscape (Pinheiro and Monteiro 2009). The garden is at 630 m of altitude, and the soil is a red latossol, under the influence of a seasonal climate with a dry winter (May–September) and wet summer (October–April). Local annual rainfall is approximately 1500 mm, and mean annual temperature is 22 °C (Cavassan et al. 1984).

### Experimental design and model development

We randomly collected fully expanded leaves from four quadrants (N, S, E, W) of each plant canopy at breast height, using 3–5 plants per species. To avoid under- or overestimation of LA, leaves with mechanical damage or partially eaten by herbivores were not included in the samples. Although using only healthy and fully expanded leaves may overestimate LA, including damaged leaves into the samples could add another factor to our model, such as the herbivory rate, which is seasonally dependent. For instance, the herbivory rate varies between seasons for *Q. parviflora* (Gonçalves-Alvim et al. 2006). In addition, LA models developed for many species consider only green, healthy, and fully expanded leaves in order to avoid uncontrolled factors into the models (Kandiannan et al. 2009; Rouphael et al. 2010; Souza and Amaral 2015).

Leaf area (LA), leaf length (L), and width (W) were measured on pooled 100 leaves per species ( $n = 500$ ), using a scanner (C4280 Photosmart; Hewlett Packard, USA) coupled to a microcomputer running an image scanning

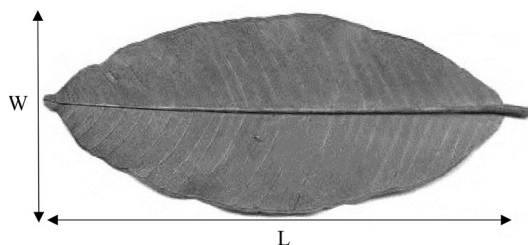
software (ImageJ for Windows—Microsoft, USA). Leaves were scanned at 300 dots per inch (dpi), and L was considered as the maximum length along the midrib and W, as the maximum width perpendicular to the midrib (Souza and Amaral 2015) (Fig. 1). Mean values, standard deviations (SD), and minimum and maximum values of LA, L, and W of each species are shown in Table 1.

Regression equations were obtained from the relationship between the dependent variable, LA, and independent variables L, W,  $L^2$ ,  $W^2$ , LW, and  $L^2W^2$  (Table 2). For two-dimensional models, involving LW and  $L^2W^2$ , the collinearity between these variables was tested using the variance inflation factor (VIF) (Marquardt 1970) and the tolerance value (T) (Gill 1986). If VIF was larger than 10 or if T was smaller than 0.10, one of the variables (L or W) should be excluded from the model.

We obtained the coefficient of determination ( $R^2$ ), mean square error (MSE), root mean square error (RMSE), and the prediction of sum of squares (PRESS). For us, the best model was represented by the combination of the highest  $R^2$  and lowest MSE, RMSE, and PRESS (Peksen 2007; Souza and Amaral 2015). We checked the variance of leaf shape (LA, L, and W) between the species by analysis of variance (ANOVA) and compared mean values by Tukey test at 5 % level.

### Validation of models

To validate the best-fitted model (highest  $R^2$  and lowest MSE, RMSE, and PRESS), additional 100 leaves from different plants of each species were collected at the same site, in September 2014. We measured LA, L, and W using the same procedures described above. The predicted leaf area (PLA) for each species was determined according to the parameters obtained from the best-fitted model selected. To test the correlation between PLA and observed LA (OLA—leaf area measured by leaf scanning and computing LA with the ImageJ software) (Peksen 2007; Souza and Amaral 2015), linear equations were used to obtain PLA for each species. Differences between OLA and PLA were plotted (Fig. 2). To estimate the relative bias, we



**Fig. 1** Schematic representation showing the position of length (L) and width (W) of a typical Vochysiaceae leaf

considered mean values  $\pm 3$  SD of the difference between OLA and PLA. If such differences are normally distributed, then 97 % of them must lie between the limits of agreement (Peksen 2007).

### Results

In the dataset for model construction ( $n = 500$ ), mean values of leaf area (LA), length (L), and width (W) differed significantly ( $P < 0.05$ ) between the species. *Q. grandiflora* showed the largest LA, while *Q. cordata* and *V. tucanorum* showed the smallest ones. As naturally expected, leaves with the largest LA, as exhibited by *Q. grandiflora*, also showed the longest and widest leaves, and in contrast (but also showing harmony between leaf dimensions), *V. tucanorum* showed the smallest LA and the narrowest leaves (Table 1). For two-dimensional models, the degree of collinearity was tested, and VIF lied between 0.01 and 0.08 and T, between 100 and 12.5, indicating that L and W can be used without collinearity.

All models presented coefficient of determination ( $R^2$ ) above 0.80, but most of the models also showed high values of MSE, RMSE, and PRESS. The model  $LA = -2.133 + 0.719 LW$  was selected because it presented the highest  $R^2$  (0.99) and the lowest MSE, RMSE, and PRESS values (Table 2).

To validate the selected model, PLA and OLA of *Q. cordata*, *Q. grandiflora*, *S. convallariodora*, *V. cinnamomea*, and *V. tucanorum* were regressed. Correlation coefficients ( $R^2 > 0.98$ ;  $P < 0.001$ ) indicated a strong correlation between OLA and PLA for every species tested (Fig. 2). Differences between OLA and PLA were normally distributed for *Q. cordata*, *Q. grandiflora*, *S. convallariodora*, *V. cinnamomea*, and *V. tucanorum*. For all species, more than 97 % of these differences lied between the limits of agreement (difference  $\pm 3$  SD) (Fig. 3).

### Discussion

To estimate LA, accepted models must possess coefficients of determination ( $R^2$ ) higher than 0.95 (Williams and Martinson 2003). In the present study, we observed  $R^2 = 0.99$  for the model we chose as presenting the best-fitted parameters. This  $R^2$  value is in accordance with other best-fitted models developed to estimate leaf area in cerrado species [*Styrax pohlii* ( $LA = 0.582 + 0.683 LW$ ,  $R^2 = 0.98$ ) and *S. ferrugineus* ( $LA = -0.666 + 0.704 LW$ ,  $R^2 = 0.97$ ) (Souza and Habermann 2014) and *V. ferruginea* ( $LA = 0.463 + 0.676 LW$ ,  $R^2 = 0.96$ ) (Souza and Amaral 2015)].

Low values of MSE, RMSE, and PRESS are also important to validate a model. Therefore, the model we

**Table 1** Mean values ± standard deviation, minimum (Min), and maximum (Max) values of leaf area (LA), length (L), and width (W) of mature leaves of Vochysiaceae plants

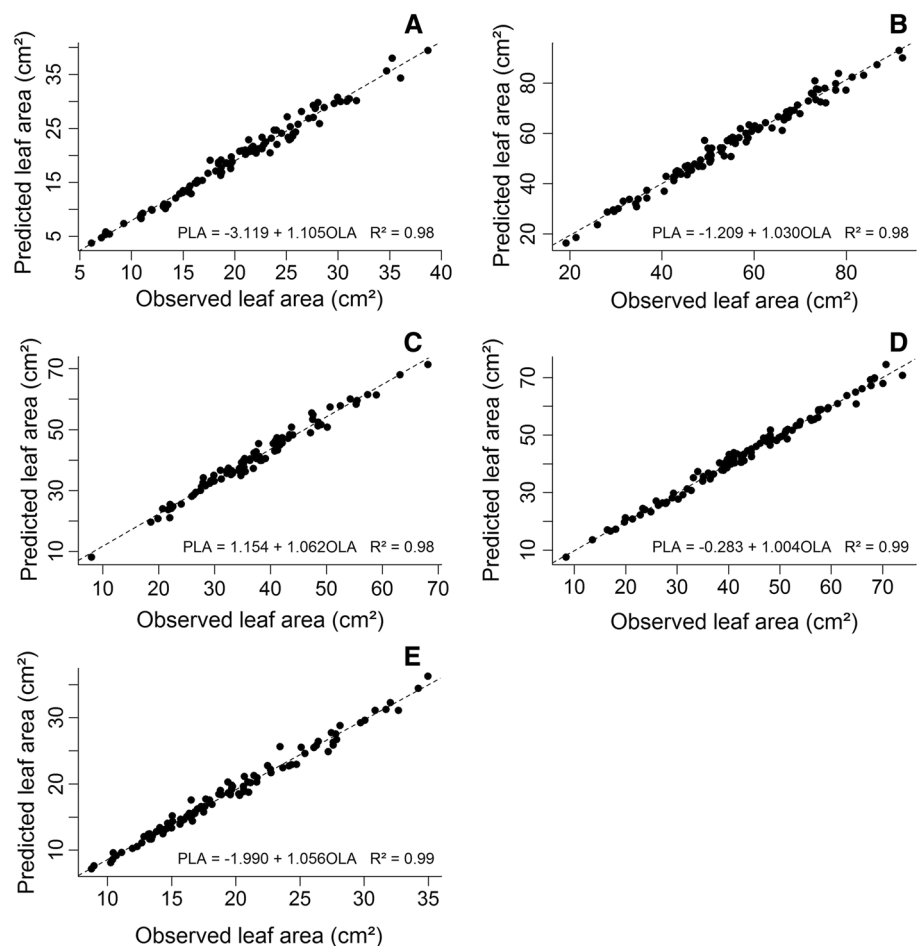
Species	Leaf area (cm <sup>2</sup> )			Leaf length (cm)			Leaf width (cm)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
<i>Qualea cordata</i>	22.02 ± 7.34	4.88	45.05	9.45 ± 2.18	3.39	13.76	3.34 ± 0.52	1.93	4.85
<i>Qualea grandiflora</i>	69.63 ± 29.11	19.20	177.96	15.78 ± 3.12	6.86	23.35	6.13 ± 1.41	3.35	10.88
<i>Salvertia convallariodora</i>	37.01 ± 10.80	7.97	68.15	13.85 ± 2.09	5.97	18.68	4.21 ± 0.64	2.38	5.47
<i>Vochysia cinnamomea</i>	43.53 ± 13.25	8.39	74.40	11.73 ± 2.10	4.88	16.45	5.26 ± 0.77	2.75	6.94
<i>Vochysia tucanorum</i>	19.90 ± 6.90	3.63	34.98	8.91 ± 2.08	2.95	13.68	3.21 ± 0.51	1.81	4.19

**Table 2** Statistics and parameter estimates from regression models for leaf area (LA) estimation

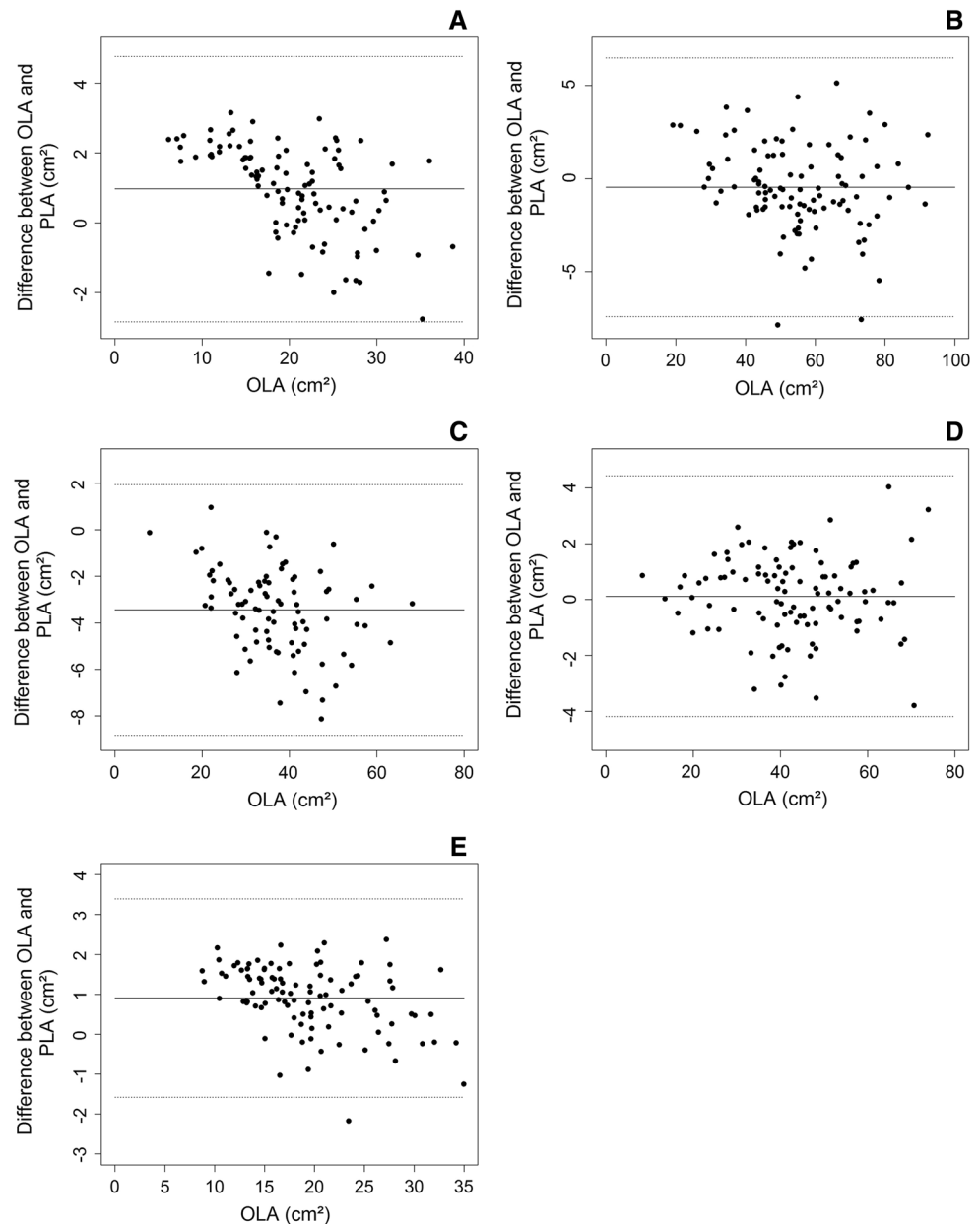
Model	Equation	MSE	RMSE	PRESS	R <sup>2</sup>
LA = α + β L	LA = -41.992 + 6.889 L	170.87	13.07	83,794.48	0.79
LA = α + β L <sup>2</sup>	LA = -2.448 + 0.274 L <sup>2</sup>	114.78	10.71	56,271.44	0.86
LA = α + β W	LA = -37.512 + 17.176 W	63.11	7.94	30,999.50	0.92
LA = α + β W <sup>2</sup>	LA = 4.658 + 1.556 W <sup>2</sup>	46.57	6.82	22,887.83	0.94
LA = α + β LW	LA = -2.133 + 0.719 LW	7.49	2.74	3,682.26	0.99
LA = α + β L <sup>2</sup> W <sup>2</sup>	LA = 0.231 + 0.022 L <sup>2</sup> W <sup>2</sup>	67.95	8.24	34,290.23	0.92

MSE mean square error, RMSE root mean square error, PRESS prediction sum of squares, and the coefficient of determination (R<sup>2</sup>) (n = 500)

**Fig. 2** Individual readings of the predicted leaf area (PLA) and observed leaf area (OLA) of *Qualea cordata* (A), *Qualea grandiflora* (B), *Salvertia convallariodora* (C), *Vochysia cinnamomea* (D), and *Vochysia tucanorum* (E) for the validation of the model  
LA = -2.133 + 0.719 LW



**Fig. 3** Individual readings of the differences between predicted leaf area (PLA) and observed leaf area (OLA) of Vochysiaceae plants versus the OLA of Vochysiaceae plants. Solid lines are the average difference between PLA and OLA, and dotted lines are the limits of agreement ( $d \pm 3SD$ ). **A** *Qualea cordata*, **B** *Qualea grandiflora*, **C** *Salvertia convallariodora*, **D** *Vochysia cinnamomea*, and **E** *Vochysia tucanorum*



selected to estimate LA of Vochysiaceae species not only possesses  $R^2 > 0.98$  but also shows MSE, RMSE, and PRESS ranging between 10 and 30 % of those presented by the other models we tested (Table 2). These conditions are also critical for accepted best-fitted models to estimate LA in *Vicia faba* (Peksen 2007), *Rosa hybrida* (Rouphael et al. 2010), and *Vernonia ferruginea* Less. (Souza and Amaral 2015).

The best-fitted model we propose ( $LA = -2.133 + 0.719LW$ ) is derived from a two-dimensional ( $LW \times LA$ ) regression. Models that were derived from a single-dimensional regression, such as  $LA = -41.992 + 6.889 L$ , exhibited the lowest  $R^2$  (0.79), while those that were derived from two-dimensional regressions showed high  $R^2$  values (Table 2). In fact, Souza and Amaral (2015)

observed that models derived from single-dimensional regressions showed  $R^2 \approx 0.40$  (for  $L$  and  $L^2$ ) and  $R^2 \approx 0.66$  (for  $W$  and  $W^2$ ). These same authors demonstrated that models derived from two-dimensional regressions exhibited  $R^2 \approx 0.96$  (for  $LW$ ) and  $0.95$  (for  $L^2W^2$ ). On the other hand, when proposing the best-fitted model to estimate LA in chestnut plants (Serdar and Demirsoy 2006) and grapevines (Williams and Martinson 2003), these authors observed that the  $R^2$  range exhibited by single- and two-dimensional derived models was not so large. Similar narrow  $R^2$  range was observed when developing a model for *V. ferruginea* (Souza and Amaral 2015). The reason for such peculiarity in the models developed for these species might be due to the ratio between  $L$  and  $W$ , which can be

considered an exception in these plants. In these cases, the lowest MSE, RMSE, and PRESS are critical to choose the best-fitted model.

Most of the models have been developed to estimate LA of single species (e.g., *Pistia stratiotes* (Carvalho et al. 2011a), *Styrax pohlii* A. DC., and *Styrax ferrugineus* Nees & Mart. (Souza and Habermann 2014), *V. ferruginea* (Souza and Amaral 2015)) and cultivars of the same species [e.g., *Vicia faba* (Peksen 2007), *Zingiber officinale* (Kandiannan et al. 2009), *Rosa hybrida* (Rouphael et al. 2010)]. In this paper, we developed a model for estimating LA of five Vochysiaceae species belonging to three different genera (*Qualea*, *Salvertia*, and *Vochysia*). Therefore, this is the first proposed model for estimating LA of multiple savanna woody species, especially in a group of Al-accumulating plants.

As Al accumulation is a trait that is fairly studied in these plants, and studies devoted to understand the function of Al in these species involve the investigation of the association of Al with sclerophyllous leaves, our model might be useful for future studies on the subject. For instance, Souza et al. (2015) noted significant variations in SLA between Al-accumulating and non-accumulating species but could not verify the direct influence of these plant functional groups on LA due to a lack of non-destructive models to use in the field. For similar reasons, leaves had to be sampled and digitized on a scanner when studying the influence of herbivory, nutrients, chemical defenses, and water status in an Al-accumulating species (Gonçalves-Alvim et al. 2006, 2011).

Therefore, the model we propose could be validated to estimate LA of *Q. cordata*, *Q. grandiflora*, *S. convallariodora*, *V. cinnamomea*, and *V. tucanorum*. The validation tests resulted in  $R^2 > 0.98$ , assuring that this model is accurate and can be used for estimating LA of these species in the field or in potted-plant experiments, using a ruler and a calculator. Consequently, future studies involving these (Al-accumulating) species may benefit from our LA model.

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