TWIG CHARACTERISTICS WITH THE SCALE LEAF AND ITS LEAF AREA ESTIMATE MODEL IN *JUNIPERUS CHINENSIS* L.

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Abstract. Leaf area measurement is particularly challenging for the scale leaf due to its tiny incomplete leaves overlapping in a decussate phyllotaxy. This study developed a non-destructive, rapid, and accurate method to measure the leaf area of a scale leaf twig. To achieve this, we used a vernier calliper to measure the length (L) and the diameter in the middle in 3 different directions of 202 twigs in *Juniperus chinensis* L. and calculated the equivalent diameter (D). Then, utilizing ImageJ and 12,612 photos of the scaly leaves on these twigs, we calculated the total leaf area (LA) and finally weighed the twig dry mass (DM) after drying. The study analyzed an estimating model for the leaf area of the twigs and the relationship between twig traits and LA using SPSS. The coefficient of variation (CV) of LA was 0.611, with a range of 67.068~79.490 mm² (95% CI). Furthermore, LA showed a significant positive correlation with both length and dry mass (r=0.949, 0.920). The optimal multivariate linear regression model for LA was: $Y = -47.841 + 2.409\,L + 19.151\,D + 1.059\,DM$ (R^2 = 0.931, RMSE = 11.808, AIC = 1265.624). The model exhibited a prediction accuracy of 90.03%. This study provides a suitable model for estimating the leaf area in *Juniperus chinensis* L.

Keywords: *non-destructive method, mathematical models, prediction accuracy, image processing*

Introduction

Leaves, as essential plant organs, facilitate photosynthesis, transpiration, and gas exchange, playing a key role in plant growth and ecosystem function (Fracasso et al., 2017; Busch et al., 2024). The leaf area directly determines the efficiency of light energy absorption and utilization by plants (Lusk, 2004). This, in turn, affects their photosynthesis, growth rate, and the carbon cycle and energy flow in ecosystems (Atsuhiro et al., 2014; Wang et al., 2019). Therefore, accurately determining leaf area is crucial when studying photosynthesis, plant adaptation, and ecological function.

The morphology of leaves varies considerably between different plant species, and the methods employed to quantify their area also differ significantly. The leaf area measurement of broad-leaved leaves is usually performed using the grid counting method, punching method, and leaf area meter measurement (Wang et al., 2020). Elongated lanceolate leaves can be measured using vernier callipers and handheld leaf area meters (Peng et al., 2021; Shen et al., 2024). Coniferous leaf area can be measured using the WinSEEDLE seed and conifer image analysis system (Daniel et al., 2013; Homolová et al., 2013). Furthermore, the above methods are usually used after leaf removed from plants. This causes significant changes of some physiological parameters following leaf removal, potentially leading to inaccuracies in physiological assessments when destructive measurement methods are employed (Yu et al., 2020). Currently, empirical models based on leaf length, leaf width, or their relationship with leaf area are often used to predict leaf area (Azeem et al., 2020; Dias et al., 2022), thus avoiding destructive sampling of leaves. For plants with tiny incomplete leaves overlapping in a decussate

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phyllotaxy, traditional methods of measuring leaf area are not readily applicable, moreover, non-destructive measurement of LA is difficult to achieve due to overlapping leaves in twigs. Thus, we need to establish a LA estimation model from the perspective of twigs.

The genus *Juniperus* is one of the most diverse genera in the Cupressaceae family, including about 75 species (Yermagambetova et al., 2022), and these species are widely distributed in almost all continental hemispheres in the North (Seca, et al., 2006). In addition to being extensively utilized in traditional medicine, *Juniperus* species play an important role in ecological and afforestation projects (Yermagambetova et al., 2022). *Juniperus* species usually have the scaled leaves, its tiny leaves are arrayed in branches in a decussate phyllotaxy, called twigs. Establishing a LA estimation model based on twigs as functional units is necessary. Thus, the length (L), equivalent diameter (D) and dry mass (DM) of twigs were measured. The LA of every twig was calculated using the image processing software ImageJ. Multivariate linear models, single-variable linear functions, exponential functions, and power functions were used to analyze their relationship between LA and parameters of twig traits. The twig parameters were selected for the optimal LA estimate model, finally established an LA estimating model for the scaly leaf twigs in *Juniperus chinensis* L. This study aimed to develop a non-destructive, rapid, and accurate method for measuring the leaf area of a scale leaf. This also provides a data basis for studying the relationship between the twig and leave trait parameters in *Juniperus chinensis* L.

Materials and methods

Field sampling

The sampling site was located on the campus of Henan University of Science and Technology (34°36′N, 112°24′E) and Zhoushan Forest Park (34°32′N, 112°16′E), China. The climate in this area is warm temperate continental monsoonal. The annual average temperature is 14.6℃, the average relative humidity is 59.6%, and the annual precipitation is 590 mm, according to local meteorological records (34°29′24"N, 112°15′36"E) from the previous ten years (2010-2019). The soil type is brown loam, and the light and hydrothermal conditions are suitable for the growth of *Juniperus chinensis* L.

A total of 202 twigs were collected from 68 *Juniperus chinensis* L. in different ages and orientations, using random sampling methods. Each twig consists of a branch and its leaves, and tiny incomplete leaves are closely arrayed in a decussate phyllotaxy with adjacent leaves partially overlapping (*Fig. 1*). The fresh mass of each twig was determined by weighing it on an electronic balance with an accuracy of 0.0001 g. The length (L), the diameter in the middle in 3 different directions, and number of leaves (K) of *Juniperus chinensis* L. twigs were measured using vernier callipers (accurate to 0.05 mm). The diameter of the twig was calculated using the formula developed by Snowdon et al. (2002): $D = \sqrt{\sum d_i^2}$ (Eq.1), where D = equivalent diameter, d_i = diameter in the middle of the twig and i = number of repetitions of the middle diameter of a twig. Using ImageJ software to process the scaly leaves images, where the images were transformed into black and white scales. The black area of the images was used to calculate the LA of each twig (Leite et al., 2021; Mielke et al., 2023). Subsequently, the sample underwent thermal treatment in an oven at 90°C for 10 minutes. Following this, the dry mass (in milligrams) was determined by drying the leaves at 80°C until a constant mass was attained. This value was then used as the measured twig dry mass (DM).

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Figure 1. *Twig in Juniperus chinensis L. (A) containing scaly leaves differing in shape and size (B). Note that scaly leaves sampled from apical (as), median (ms), and basal (bs) portions of the same twig differ in shape and size*

Construction of empirical models

The empirical model of LA was constructed using a random sample of 80% of the data, with the remaining 20% used to evaluate the model's accuracy (Carvalho et al., 2017; Azeem et al., 2020). To investigate the relationship between different twig trait parameters and LA, a multivariate linear model was employed: $Y = \mathrm{a} + \sum b_i X_i, i = 1 \sim 4$. We used the stepwise method to optimize the empirical model. Further investigation was conducted to estimate LA using a single trait parameter. Based on the distribution of scatter plots, we employed three types of models to fit the data: linear: $y = ax + b$, exponential: $\mathrm{y} = ae^{bx}$ and power functions: $\mathrm{y} = ax^b$. The measured LA was the dependent variable (y), the twig trait parameters were the independent variable (x), and the empirical model's coefficients (a and b) were computed (Toebe et al., 2019; Sabouri et al., 2021). We used the LA fitting area model and resolved the equation coefficients using the weighted least squares approach. The equation parameters and graphics were performed using SPSS and Origin. The variance inflation factor (VIF) was used to diagnose collinearity when building the multivariate linear model. Collinearity was diagnosed using the formula developed by Marquardt et al. (1970): $VIF = 1/(1 - r^2)$ (Eq.2), where r is the correlation coefficient between the variables. A VIF value exceeding 10 indicates significant multicollinearity between variables, and at least one variable must be excluded when constructing the model. If the VIF value is less than 10, it indicates that the multicollinearity problem is irrelevant and these two parameters can be retained when constructing the model.

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Determination of the optimal empirical model

Similar to what Ribeiro et al. (2020) and Dias et al. (2022) reported, the optimal estimation model was comprehensively identified based on Akaike Information Criterion (AIC), incorporating the coefficient of determination (R^2) and root mean square error (RMSE). Furthermore, this study clarifies that AIC values are prioritized for selecting the optimal empirical model. AIC was calculated using the formula developed: $AIC = 2K + n\left[\ln 2\Pi \frac{\sum_{i=1}^{n}(y_i - f_i)^2}{n} + 1\right]$ (Eq.3). When the AIC difference between the top two empirical models is less than 2, the model with the smaller RMSE value is selected as the optimal empirical model. RMSE was calculated using the formula developed: $RMSE = \sqrt{\sum_{i=1}^{n} \frac{(y_i - f_i)^2}{n-1}}$ (Eq.4). The optimal empirical model for LA is then determined based on these rules (Wu et al., 2021).

We calculated the predicted value of LA using the optimal empirical model. Subsequently, the distribution of residuals was analyzed. When the residual distribution approximates normality and the majority of residual points reside within the range of the mean residual ± 3 standard deviations, the empirical model is considered to be both reliable and reasonable (Chiang et al., 2003).

Evaluation of the optimal empirical model

To further evaluate the reliability of the selected empirical model in more depth, the remaining 20% of the data and the optimal empirical model were used to obtain the predicted value of LA, and then regression analysis was performed on the predicted and measured values of LA. To assess the consistency between the predicted and measured values, the slope of the regression line was determined and evaluated based on its proximity, the intercept size, and the coefficient of determination (R^2). Calculation of R^2 using the formula developed: $R^2 = \frac{1}{n}\sum_{i=1}^{n} 1 - abs\frac{(y_i - f_i)}{y_i} \times 100\%$ (Eq.5). Additionally, the prediction accuracy (FA) of the optimal empirical model was calculated. Calculation of FA using the formula developed: $FA = \frac{1}{n}\sum_{i=1}^{n}\left[1 - abs\frac{(y_i - f_i)}{y_i} \times 100\%\right]$ (Eq.6). (Wu et al., 2021).

K represents the number of parameters in the empirical model, whereas *n* denotes the number of samples. The LA value of the i_{th} sample, denoted by y_i, serves as the dependent variable, while the LA prediction value of the i_{th} sample, denoted by f_i, serves as the independent variable. The absolute value function, represented by *abs*, is utilized to calculate the correlation coefficient.

Data analysis

Linear regression analysis was employed to construct models based on the twig traits and leaf area. Pearson correlation analysis was conducted to determine the relationship between leaf area and twig traits. All statistical analysis and model fitting were performed using IBM SPSS Statistics (version 22), while Microsoft Excel 2016 was used to record and organize the data.

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Results

Trait parameters of scaly leaf twigs are statistically examined in *Juniperus chinensis* L. (*Table 1*). The results indicate that among 202 twig samples (12,612 scaly leaves), the variation coefficient of DM was the largest (CV=0.700) in the twig trait parameters in *Juniperus chinensis* L., followed by LA, L, K, and D. The mean value of DM was 14.446 ± 10.110 mg (mean ± standard deviation, as indicated below), with a numerical range of 13.043~15.848 mg (95% CI). The coefficients of variation for LA and L are 0.611 and 0.517, respectively, with average values of 73.279 ± 44.768 mm² and 23.674 ± 12.228 mm, and numerical ranges of 67.068-79.490 mm² (95% CI) and 21.977-25.370 mm (95% CI). The variation coefficient of K was low (CV=0.438), with a mean value of 62.436 ± 27.321 mm, and a numerical range of 58.645~66.226 units (95% CI). The variation coefficient for D was the lowest (CV=0.135), with a mean value of 2.567 ± 0.348 mm and a variation range of 2.519~2.616 mm (95% CI). Its numerical distribution was closest to the mean (SE=0.024, MAD=0.276), which suggests that D has the least amount of numerical fluctuation and deviation.

Table 1. *Overall distribution characteristics of scaly leaf twig trait*

Statistics	Measurements				
	L/mm	**D/mm**	**K/pcs**	**LA/mm²**	**DM/mg**
Sample size	202	202	202	202	202
Mean	23.674	2.567	62.436	73.279	14.446
Standard error of mean	0.860	0.024	1.922	3.150	0.711
Standard deviation	12.228	0.348	27.321	44.768	10.110
Upper 95% confidence interval（CI）of mean	25.370	2.616	66.226	79.490	15.848
Lower 95% confidence interval（CI）of mean	21.977	2.519	58.645	67.068	13.043
Minimum	3.720	1.622	14.000	6.319	0.500
Maximum	48.017	3.449	125.000	171.818	44.600
Coefficient of variation	0.517	0.135	0.438	0.611	0.700
Skewness	0.185	-0.471	0.175	0.372	0.647
Kurtosis	-1.060	-0.122	-0.765	-1.006	-0.450
Mean absolute deviation	10.412	0.276	23.001	38.547	8.475

Note: LA - twig leaf area; DM - twig dry mass; L - twig length; D - twig equivalent diameter; K - number of leaf

As shown in *Table 2*, LA has a significant correlation with DM, L, K, and D in *Juniperus chinensis* L., Among them, the correlation coefficients between LA and DM, as well as between LA and L, are the highest (0.949 and 0.920, respectively), while the correlation coefficient between D and LA is the lowest (r=0.664). Therefore, these twig trait parameters can be used as potential parameters for estimating leaf area.

Table 2. *Correlation of scaly leaf twig trait parameters*

Index	L/mm	D/mm	K/pcs	DM/mg
L/mm				
D/mm	0.548**			
K/pcs	0.920**	0.464**		
DM/mg	0.888**	0.651**	0.789**	
LA/mm²	0.949**	0.664**	0.857**	0.920**

Note: ** represents a significant difference at P=0.05; L - twig length; D - twig equivalent diameter; K - number of leaves; DM - twig dry mass; LA - twig leaf area

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Multivariate linear fitting was used to further analyze the relationship between LA and other twig trait parameters (L, D, K and DM). Firstly, the all-in method was used to construct a multivariate linear regression equation model (*Table 3*): $Y = -47.620 + 2.438X_1 + 19.132X_2 + 1.057X_3 - 0.013X_4$ (R^2 = 0.931, RMSE = 11.807, AIC = 1267.602), where X_1 represents L, X_2 represents D, X_3 represents DM, and X_4 represents K. Although the fit of Model 1 was appropriate (R^2 = 0.931), the regression coefficient of K in Model 1 was not statistically significant (P = 0.884), and the multicollinearity among the four variables could not be ignored. Therefore, a stepwise approach is recommended to optimize the process. In Model 2, L is the independent variable, and the equation for Model 2 is as follows: $Y = -9.331 + 3.523X_1$ (R^2 = 0.893, RMSE = 14.793, AIC = 1334.646). Based on Model 2, the variable D is introduced to obtain Model 3: $Y = -65.405 + 3.090X_1 + 25.741X_2$ (R^2 = 0.921, RMSE = 12.673, AIC = 1286.525), where X_1 represents L and X_2 represents D. Variable DM is introduced to get Model 4: $Y = -47.841 + 2.409X_1 + 19.151X_2 + 1.059X_3$ (R^2 = 0.931, RMSE = 11.808, AIC = 1265.624), where X_1 represents L, X_2 represents D, and X_3 represents DM. Model 4 achieved the optimal fit with the three variables (R^2 = 0.931, RMSE = 11.808, AIC = 1265.624) and was considered the optimal multivariate regression model.

Table 3. *Multivariate linear regression of scaly leaf twig trait parameters predicted leaf area*

Methods	Model $Y = \mathbf{a} + \sum b_i X_i$, $i = 1-4$	Independent Variable X_i	Coefficient	Standard Error	Significance	VIF	95% Confidence Interval	
							Lower	Upper
Complete substitution method	$i = 1$	Constant	-47.620	8.209	<0.001		-63.834	-31.406
	$R^2 = 0.931$	L	2.438	0.258	<0.001	11.101	1.929	2.946
	$RMSE = 11.807$	D	19.132	3.456	<0.001	1.750	12.306	25.958
	$AIC = 1267.602$	DM	1.057	0.218	<0.001	5.623	0.626	1.487
		K	-0.013	0.088	0.884	6.328	-0.187	0.161
Stepwise method	$i = 2$	Constant	-9.331	2.544	<0.001		-14.355	-4.308
	$R^2 = 0.893$	L	3.523	0.096	<0.001	1.000	3.334	3.713
	$RMSE = 14.793$							
	$AIC = 1334.646$							
	$i = 3$	Constant	-65.405	7.702	<0.001		-80.615	-50.194
	$R^2 = 0.921$	L	3.090	0.100	<0.001	1.480	2.892	3.288
	$RMSE = 12.673$	D	25.741	3.390	<0.001	1.480	19.045	32.436
	$AIC = 1286.525$							
	$i = 4$	Constant	-47.841	8.042	<0.001		-63.725	-31.956
	$R^2 = 0.931$	L	2.409	0.168	<0.001	4.730	2.078	2.740
	$RMSE = 11.808$	D	19.151	3.443	<0.001	1.747	12.352	25.950
	$AIC = 1265.624$	DM	1.059	0.216	<0.001	5.578	0.632	1.487

Note: L - twig length; D - twig equivalent diameter; K - number of leaves; DM - twig dry mass; LA - twig leaf area; R^2 - Adjusted R-Square; RMSE - Root Mean Square Error; AIC - Akaike Information Criterion

To explore the influence of univariate factors on LA estimation, three types of models, linear function $y = ax + b$, exponential function $y = ae^{bx}$ and power function $y = ax^b$. were used for fitting examination according to the distribution of scatter plots of each twig trait parameter (*Table 4*). The four parameters of the twig trait in *Juniperus chinensis*

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L. were highly significant with LA (P < 0.001), and the R^2, RMSE, and AIC values of each fitting model ranged from 0.454 to 0.928, 14.793 to 40.278, and 1334.646 to 1659.179, respectively. Among the three fitting models, the optimal estimation model for LA was based on L: $Y = -9.331 + 3.523X$ (R^2=0.893, RMSE=14.793, AIC=1334.646); $Y = 14.412e^{0.059X}$ (R^2=0.844, RMSE=26.453, AIC=1522.961); $Y = 1.654X^{1.187}$ (R^2=0.928, RMSE=14.914, AIC=1337.289). Therefore, the linear model between LA and L is the optimal univariate empirical model.

Table 4. *Univariate regression model of scaly leaf twig trait parameters predicted leaf area*

Model	Independent Variable X_i	Parameters			Statistics			
		a	b	N	R^2	RMSE	AIC	P
Linear function $y = ax + b$	L	3.523	-9.331	162	0.893	14.793	1334.646	<0.001
	D	85.221	-145.833	162	0.454	33.450	1598.990	<0.001
	DM	4.042	14.773	162	0.832	18.573	1408.372	<0.001
	K	1.445	-16.287	162	0.731	23.474	1484.239	<0.001
Exponential function $y = ae^{bx}$	L	14.412	0.059	162	0.844	26.453	1522.961	<0.001
	D	1.053	1.552	162	0.515	35.702	1620.099	<0.001
	DM	22.289	0.065	162	0.730	40.278	1659.179	<0.001
	K	11.878	0.025	162	0.765	33.629	1600.720	<0.001
Power function $y = ax^b$	L	1.654	1.187	162	0.928	14.914	1337.289	<0.001
	D	1.654	3.791	162	0.521	34.676	1610.652	<0.001
	DM	8.673	0.803	162	0.870	17.654	1391.928	<0.001
	K	0.183	1.430	162	0.852	24.587	1499.251	<0.001

Note: N - Number of points; L - twig length; D - twig equivalent diameter; K - number of leaves; DM - twig dry mass; LA - twig leaf area; R^2 - Adjusted R-Square; RMSE - Root Mean Square Error; AIC - Akaike Information Criterion; P - significant difference

The residual plots of the LA estimates fitted by the two selected models show a normal distribution (*Fig. 2*), and all residual points are distributed within the range of the mean residual ± 3 standard deviations. This also is preliminary evidence of the reliability of the optimal LA empirical model constructed in this study for predicting leaf area.

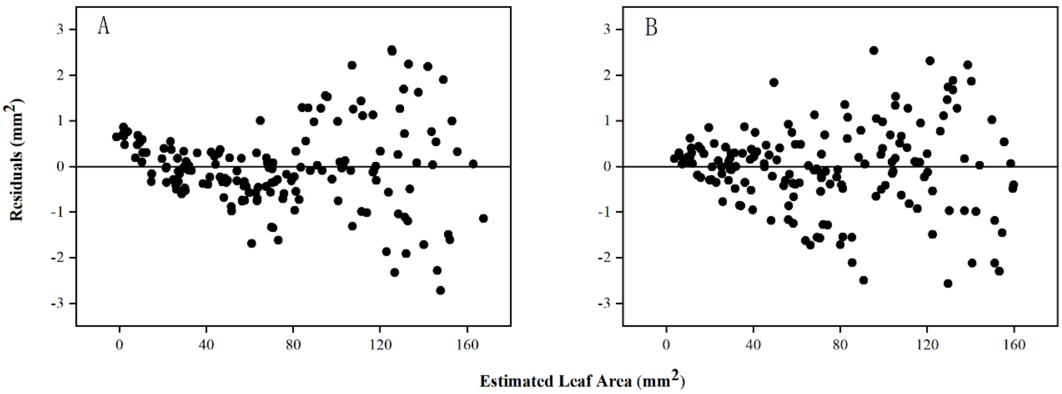

Figure 2. *Analysis of the standardized residual distribution of scaly leaf twigs LA estimated using optimal multivariate and optimal univariate regression models. A: Multivariate regression equation model; B: A linear model based on L. Data were obtained from 162 twigs (10078 scaly leaves)*

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To further evaluate the reliability of these optimal empirical models, we used the remaining 20% of the data to calculate the predicted scaly leaf twigs LA in *Juniperus chinensis* L. based on the selected optimal model. *Fig. 3* illustrates the regression equation that was created using the predicted value as y and the measured value of LA as x. The regression analysis equation between the LA predicted values and the measured values of the two models is close to the $y = x$ linear equation, which is represented by the dashed line in the image. The multivariate linear model's and univariate linear model's coefficients of determination (R^2) between the LA predicted and LA measured values are 0.985 and 0.940 (Eq.5), respectively. Here, the multivariate linear model displays a better fit. The optimal multivariate linear model (Model 4) and the univariate linear model based on L for LA have prediction accuracy of 90.03% and 83.99% (*Eq.6*), respectively. These results show that both models can accurately predict scaly leaf twigs LA in *Juniperus chinensis* L., but the multivariate linear model has higher accuracy.

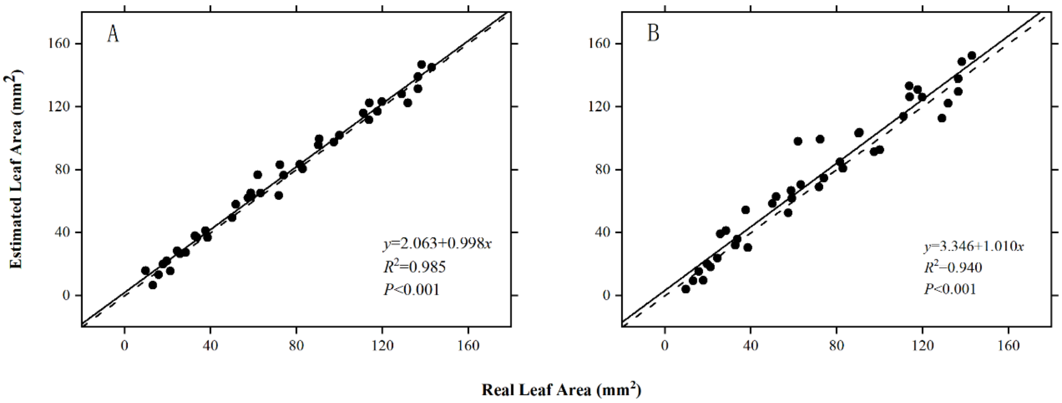

Figure 3. *Validation of regression models adjusted to estimate scaly leaf twigs LA. A: Multivariate regression equation model; B: A linear model based on L. Validation was performed by evaluating the relationship between real leaf area (independent variable) and estimated leaf area (dependent variable). Data were obtained from 40 twigs (2534 scaly leaves)*

Discussion

Characteristics of scaly leaf twigs

The study analyzed the variation feature of 202 twig traits. With coefficients of variation of 0.517 and 0.135, respectively, L had higher variability than D, while the coefficient of variation for LA was up to 0.611 (*Table 1*). Similar research also confirmed that LA is more variable than leaf length and width in leaf traits (Carvalho et al., 2017; Toebe et al., 2019). We found a significant and positive association (r=0.949) between L and LA in the scaly leaf twig trait characteristics of *Juniperus chinensis* L. (*Table 2*). It is reasonable to assume that the intrinsic variability of the twig length accounts for the majority of the LA variability in *Juniperus chinensis* L. This result is consistent with a prior study conducted on the spiny leaves of *Juniperus chinensis* cv. Pyramidalis, which discovered that leaf length rather than leaf width has an important influence on LA (Wang et al., 2023). By constructing the optimal multivariate linear regression model, it was found that D had a significant influence on LA with a higher regression coefficient (19.151). And both have a significant and positive correlation (r=0.664) in the twig of *Juniperus chinensis* L. (*Table 2*), Similarly, LA was affected by L and D (Ribeiro et al.,

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2019; Zhang, 2020; Silva et al., 2023). Thus, twig shape significantly influenced its LA. The results showed that the 95% confidence interval (CI) for LA ranged from 67.068 to 79.490 mm², with a considerable standard deviation (SD = 44.768). Significant standard deviation is ascribed to the random selection of twigs, which included diverse twig ages, tree ages, canopy positions (sun-exposed and shaded twigs) and habitat. This implied that the study's sample captured the spectrum of twig trait variation in its native habitat and that the twig trait parameters were accurately measured in *Juniperus chinensis* L. In contrast to LA and L, the variation range of D, DM, and K is concentrated in the low standard deviation. It's interesting to note that L and LA have a strong correlation. These results provide compelling evidence for the reliability of the model.

Estimation model for the leaf area of scaly twigs

The multivariate linear model revealed that L and D exert the most significant influence on LA. Given this, the optimal multivariate regression model for LA was found in this study, and its prediction accuracy was up to 90.03%. Utilizing the multivariate linear model will well estimate the LA of scaly twigs in *Juniperus chinensis* L. due to its high prediction accuracy. Similarly, the estimation metrics is to use leaf width and length to establish a multivariate linear model (Goergen et al., 2021; Ribeiro et al., 2022; Sautchuk et al., 2024). However, the addition of variables complicates the computation during the establishment of the multivariate linear model. Three different univariate models-a linear function $y = ax + b$, an exponential function $y = ae^{bx}$, and a power function $y = ax^b$-were fitted to create a more straightforward LA estimation model. With a prediction accuracy of 83.99%, the linear model based on L was the optimal univariate model, namely, $Y = -9.331 + 3.523X$ ($R^{2=}$0.893, RMSE=14.793, AIC=1334.646). Scaly twigs in *Juniperus chinensis* L. are very similar to slender leaves in morphology, which are both narrow and elongated in shape. Using leaf length as the most accurate parameter in the estimation model for the LA of slender leaves (Donato et al., 2020; Peng et al., 2021; Sabouri et al., 2021). Therefore, a univariate linear model offers an easy-to-use and reliable estimation. We can use it to determine leaf area using a non-destructive method accurately.

A linear relationship was observed between the real LA and estimated leaf area by multivariate linear regression model: $Y = -47.841 + 2.409\,L + 19.151\,D + 1.059\,DM$ ($R^2 = 0.931$, RMSE = 11.808, AIC = 1265.624) among the 40 twigs (*Fig. 3*). According to Pompelli et al. (2012) and Sousa et al. (2024), even though the models generated with a linear dimension appeared to be good fits, in general these models showed biased estimates, particularly in large differences in leaf size, with errors not adjusting to a normal distribution. In this study, estimating LA can use the multivariate linear regression model based on L, D, and DM, the results showed well-distributed residue without trends biased (*Fig. 2*). When the linear coefficient exceeds zero, it has a high correlation and R^2, low RMSE and AIC, good residual distribution, and high accuracy. We recommended using a multivariate linear model $Y = -47.841 + 2.409\,L + 19.151\,D + 1.059\,DM$ to estimate the LA of scaly twigs in *Juniperus chinensis* L.

Perspectives and limitations

Previous scholars used AIC and RMSE as the main references to determine the optimal model (Ribeiro et al., 2020; Dias et al., 2022). In contrast, this study delineated a specific

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approach whereby the model with the smallest AIC value was identified as the optimal empirical model, and the model with the smaller RMSE value was selected as the optimal empirical model when the difference in AIC between the top two empirical models selected as optimal was less than two. Nevertheless, we have chosen only one species from the genus *Juniperus*, but including more tree species in future studies could enhance the accuracy of our model.

Conclusions

Based on the test and evaluation of the optimal LA estimation model, we conclude that the optimal multivariate linear regression model for LA is: $Y = -47.841 + 2.409\,L + 19.151\,D + 1.059\,DM$ (R^2 = 0.931, RMSE = 11.808, AIC = 1265.624). The optimal univariate linear model for LA is $Y = -9.331 + 3.523\,L$ (R^2=0.893, RMSE=14.793, AIC=1334.646). The prediction accuracy of the two models is 90.03% and 83.99%, respectively. While the multiple linear model has higher prediction accuracy, the univariate model provides a simpler estimation method. Given that non-destructive measurement of the leaf area of the scaly leaf twig is difficult using traditional methods, this study proposes a reliable method for estimating leaf area using multivariate linear models and univariate models based on digital photography and size measurement using ImageJ software. It also provides an important reference for estimating leaf area when tiny leaves are densely arrayed on branches. Therefore, establishing a leaf area estimation model is an effective method for non-destructive determination of leaf area in the future.

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