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Leaf shape alters the coefficients of leaf area estimation models for *Saussurea stoliczkai* in central Tibet

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Abstract

Nondestructive methods to estimate individual leaf area (LA) accurately, by leaf length (L) and/or width (W), is helpful for the *in situ* and successive LA measurements. However, leaf shape and size may covary with environment and thus alter the coefficients of LA estimation models. To test such hypothesis, we carried out an experiment by measuring *Saussurea stoliczkai* C. B. Clarke leaves along an altitudinal transect in Damxung county, central Tibet. In July 2011, we selected seven sites at about every 150 m in altitude from 4,350 m to 5,250 m a.s.l. A total of 1,389 leaves (182 to 203 leaves for each site) were measured. For each site, models developed by two leaf dimensions [LA = a (L×W) + b] could estimate LA more accurately than those by single dimension. L, W, LA and leaf shape index (L:W ratio) all decreased with increasing altitude, leading to significant differences in coefficients of two-dimension model between almost every two sites. Accordingly, a common two-dimension model is unlikely to occur for *S. stoliczkai* across the whole altitudinal transect, indicating that the varying leaf shape may alter the coefficient of LA estimation models.

Additional key words: alpine grassland; altitude; leaf area; leaf length and width; nondestructive methods.

Introduction

Leaves are the most important organs of terrestrial plants undertaken gas exchange and carbon assimilation. Leaf area (LA) strongly affects light interception, plant growth and productivity from a single plant to a whole ecosystem, and is broadly used as a key attribute for physiological, agronomic and ecological studies. However, accurate LA measurement of a large number of leaves, especially in the field, is laborious, and usually destructive (Beerling and Fry 1990). Thus, nondestructive method for leaf area estimation using leaf length (L) and/or width (W) was widely applied in many cultivated fruits (Demirsoy and Demirsoy 2003, Demirsoy *et al.* 2004, 2005; Cittadini and Peri 2006, Fallovo *et al.* 2008, Mendoza-de Gyves *et al.* 2008, Demirsoy and Lang 2010, Mazzini *et al.* 2010, Rouphael *et al.* 2010a), vegetables (Salerno *et al.* 2005, Rouphael *et al.* 2006, Peksen 2007, Rivera *et al.* 2007, Tsialtas and Maslaris 2008, Kandiannan *et al.* 2009, Olfati *et al.* 2010), ornamental crops (Rouphael *et al.* 2007, 2010b; Fascella *et al.* 2009, Giuffrida *et al.* 2011) and other plants (Serdar and Demirsoy 2006, Cristofori *et al.* 2007, 2008; Antunes *et al.* 2008, Kumar 2009, Zhou and Shoko 2009, Kumar and Sharma 2010). However, only a few studies have applied this nondestructive method to estimate LA of wild species (Zhang and Liu 2010, Zhang and Pan 2011). Generally, the indirect, nondestructive method can predict accurate LA estimates, and is helpful for the *in situ* and successive LA measurements (Beerling and Fry 1990, Demirsoy 2009), therefore provides a vital role in monitoring plant growth for long-term observation.

As one of the plant morphological traits, leaf area is determined by a combination of gene action and environmental effects (McDonald *et al.* 2003, Hovenden and Vander Schoor 2006, Yates *et al.* 2010). Most of the above-mentioned studies concerned about the effects of different genotypes on LA estimation, but only a few concentrated on environmental effects (Rouphael *et al.* 2006, Serdar and Demirsoy 2006, Mendoza-De Gyves *et*

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Abbreviations: *ANCOVA* – analysis of covariance; L – leaf length; LA – leaf area; MSE – mean square errors; T – tolerance value; VIF – variance inflation factor; W – leaf width.

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al. 2007, Demirsoy and Lang 2010). There is still limited knowledge about altitudinal effects on LA estimates by using leaf dimensions (Mendoza-de Gyves *et al*. 2008). As altitude increases, individual LA of the same plant tends to decrease (Cordell *et al*. 1998, Kao and Chang 2001). However, we are not sure whether the varying leaf size would alter the coefficients of LA models, since leaf shape (ratio of L to W) may change with leaf size in some species (Rouphael *et al*. 2006).

Saussurea stoliczkai, an endemic species in Tibetan Plateau, spreads extensively across altitudes ranging from 4,300 m to 5,300 m on south-facing slopes of Nyaiqentanglha Mountains, providing us an ideal material to investigate how plants adapt to the severe high-altitude environment. Thus, the tasks of this study are: (*1*) to develop LA estimation models for *S. stoliczkai* by L and/or W for each site, and (*2*) to test if the variation in leaf shape alters the coefficients of the models along an altitudinal transect. The second task determines if a common model can be applied for LA estimation across the whole transect.

Materials and methods

Study site and species: This study was carried out on a south-facing slope of Nyaiqentanglha Mountains from 4,350 m to 5,250 m (30°30′–30°32′N, 91°03′E) near Damxung county, central Tibet. The summit of the slope is about 5,600 m. According to the meteorological measurements at Damxung county station (about 4 km away from our study site, 4,288 m), multi-annual (1963–2006) precipitation and mean air temperature is 479 mm and 1.7°C, respectively. With increasing altitude, growing season (from May to September) mean air temperature decreased from 9.58°C (4,350 m) to 3.70°C (5,250 m), but growing season precipitation increased from 361 mm (4,350 m) up to 544 mm (*ca*. 5,100 m) and then decreased.

S. stoliczkai, belonging to Asteraceae, occurs in alpine grassland as a company species. It is a perennial herb, 2–8 cm tall, with 3–8 rosette leaves which are annually wilted in winter. A typical mature leaf, with a very short petiole, is lobed and long-elliptic to oblanceolate in shape (Fig. 1), and generally 2–10 cm in length and 0.5–2.5 cm in width. The flower stage ranges from August to October.

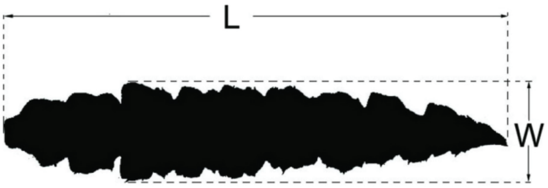

Fig. 1. Leaf length (L) and width (W) measurements of a typical mature *Saussurea stoliczkai* leaf.

Sampling and leaf measurements: In middle July of 2011, we selected seven sites at about every 150 m in altitude along the transect from 4,350 m to 5,250 m. At each site, 182 to 203 fully expanded and intact leaves were randomly sampled and enclosed in plastic envelopes immediately. In total, 1,389 leaves were collected for predicting LA. Leaf length (L) and width (W) were measured to the nearest millimeter at the same day. Here, L is the maximum value along the midrib, while W is the maximum value perpendicular to the midrib (Fig. 1). The actual one-side LA was determined with a portable area meter (*CI-203*, *CID, Inc*., USA).

Model building: In order to test the relationships between LA and L and/or W, we developed seven common regression (linear and power) models. The dependent variable is LA, while the independent variables included L, W, L^2, W^2 and the products of L and W (L×W). We first developed models using data from each site. Then, we tested the internal validity using coefficients of determinations (r^2), mean square errors (MSE) and predicted residual error sum of squares (PRESS). The best model for each altitude was selected according to the combination of the highest r^2, the lowest MSE and PRESS, and the closest values between PRESS and error sum of squares (SSE) (Rouphael *et al*. 2010b). And then, we tested the differences in the coefficients of models (slopes and intercepts) using analysis of covariance (*ANCOVA*). The differences in slopes were tested, and only if the differences in slopes were not significant ($p>0.05$), tests of differences of intercepts were then performed. Furthermore, if L and W were both involved in a model, we needed to detect the colinearity between the two parameters by calculating the variance inflation factors (VIF) (Marquardt 1970) and the tolerance value (T) (Gill 1986):

$$\mathrm{VIF} = 1/(1 - r^2)$$
$$\mathrm{T} = 1/\mathrm{VIF}$$

where r is the correlation coefficient. If the VIF value was lower than 10 or the T value was higher than 0.10, the colinearity between the two dimensions could be neglected and both parameters could be included in the model.

Regressions between LA and leaf dimensions of L and/or W, and *ANCOVA* were performed using *SPSS 13.0* package (*SPSS Inc*., Chicago, USA). Calculations of MSE, PRESS and SSE were conducted with *R 2.14.1* (*R Development Core Team*).

Results and discussion

L, W, and LA across the transect ranged from 1.8 to 9.7 cm, 0.6 to 2.9 cm, and 1.0 to 9.1 cm^2 (from Fig. 2), respectively. As altitude increased, both L (Fig. 2*A*) and W (Fig. 2*B*) decreased significantly. The decreasing rate of L (4.2 cm km^{-1}) along our transect was much higher than that of W (0.2 cm km^{-1}) (Fig. 2*A*,*B*), leading to significant decreasing trend of L:W ratio with rising altitude (Fig. 2*C*). Since both leaf dimensions decreased, LA tended to decline towards higher altitude (Fig. 2*D*).

Since L, W, L:W ratio, and LA significantly decreased with increasing altitude (Fig. 2), we first developed LA estimation models for each site (Table 1). When one parameter (L or W) was included in the models, r^2 ranged from 0.52 to 0.87, MSE from 0.096 to 0.530 and PRESS from 20.106 to 108.856 (models 1–6 in Table 1), indicating that L or W singly could not predict LA accurately. When both leaf dimensions of L and W were involved in the models (models 7 in Table 1), it exhibited the highest r^2 (0.92–0.96), the lowest MSE (0.029–0.170) and PRESS (5.540–36.122). According to the criteria for model selection (higher r^2, lower MSE, lower PRESS and closer values between PRESS and SSE), we chose model 7 as the best models for each altitude. For each site, we analyzed the degrees of colinearity between L and W for model 7. Because the VIF values ranged from 1.20 to 2.78 (<10) and the T values from 0.36 to 0.83 (>0.1), the collinearity between the two parameters could be considered negligible and both variables could be included in the models.

Although model 7 provided accurate LA estimates for each site, we would like to know if there exists a common model for this species across the whole transect. According to Table 1, however, we found the slope of model 7 tended to increase towards higher altitude (from 0.481 to 0.619). Further analysis of *ANCOVA* was performed to test the differences in slopes and intercepts of models between every two altitudes. For all pairs, differences in slopes were significant ($p<0.05$) except between 4,350 m and 4,500 m ($p=0.24$), 4,500 m and 4,650 m ($p=0.30$), 4,650 m and 4,800 m ($p=0.17$) and 5,100 m and 5,250 m ($p=0.13$). So, we further tested the differences in intercepts and found that only those of the lowest (4,350 m and 4,500 m) and the highest (5,100 m and 5,250 m) two sites did not differ ($p>0.05$). The results indicated that a common two-dimension model is unlikely to occur for this species across the whole altitudinal transect.

This study, however, is inconsistent with our previous study, in which we found a common model (either two-dimension or only leaf width) could be employed to estimate LA for an understory herb across the whole elevation range at a timberline ecotone (Zhang and Liu 2010). The disagreement might be due to the different relationships between leaf shape (L:W ratio) and altitude, which is negative in this study (Fig. 2*C*) but no significant correlation can be found in Zhang and Liu (2010). Therefore, it must be leaf shape that altered the coefficient of the two-dimensional LA estimation models along the altitudinal transect. Since leaf shape were controlled by genotype as well as environmental conditions (McDonald *et al*. 2003, Hovenden and Vander Schoor 2006, Yates *et al*. 2010), researchers must pay attention to the variation of leaf shape when estimating species-specific LA by measuring L and W, especially under the condition that the leaves were collected in different environments.

Across the transect, mean L varied for one fold while W varied for only 0.1 fold, leading to significant decreasing trend of LA and L:W ratio, consistent with previous studies (*e.g*. Körner *et al*. 1983, Halloy and Mark 1996). Since temperature decreases and precipitation increases with increasing altitude, declining LA and L:W ratio can be regarded as strategies for adapting to

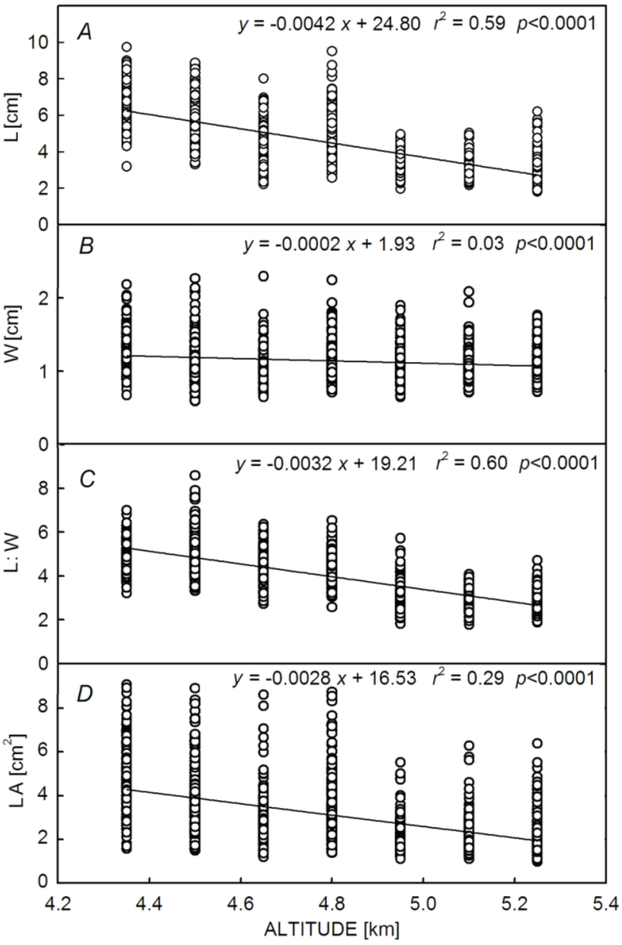

Fig. 2. Altitudinal variations in *A*: leaf length (L), *B*: leaf width (W), *C*: the ratio of leaf length to width (L:W), and *D*: leaf area (LA). Sample size is 201 for 4,350 m, 201 for 4,500 m, 182 for 4,650 m, 200 for 4,800 m, 200 for 4,950 m, 203 for 5,100 m and 202 for 5,250 m.

Table 1. Parameters (*a* and *b*), coefficients of determinations (r^2), mean square errors (MSE, cm^2), predicted residual error sum of squares (PRESS), and error sum of squares (SSE) of the models estimating the leaf area (LA, cm^2) of *Saussurea stoliczkai* from leaf length (L, cm) and/or width (W, cm). Sample size is 201 for 4,350 m, 201 for 4,500 m, 182 for 4,650 m, 200 for 4,800 m, 200 for 4,950 m, 203 for 5,100 m and 202 for 5,250 m.

Models	Sites [m]	Parameters *a*	*b*	r^2	MSE	PRESS	SSE
1 LA = *a* L + *b*	4,350	1.180	–2.907	0.81	0.395	81.138	79.315
	4,500	1.209	–3.075	0.76	0.530	108.856	106.504
	4,650	0.953	–1.576	0.75	0.363	68.916	66.114
	4,800	1.151	–2.225	0.86	0.315	64.741	63.066
	4,950	1.002	–0.969	0.55	0.229	47.083	45.704
	5,100	1.270	–1.739	0.72	0.161	33.886	32.572
	5,250	1.116	–1.224	0.80	0.154	32.050	31.098
2 LA = *a* W + *b*	4,350	5.127	–2.060	0.77	0.472	97.608	94.858
	4,500	4.721	–1.560	0.82	0.396	81.899	79.607
	4,650	4.568	–1.912	0.81	0.277	51.929	50.373
	4,800	5.289	–2.502	0.83	0.370	76.471	74.085
	4,950	2.688	–0.692	0.74	0.131	27.093	26.263
	5,100	3.314	–1.340	0.82	0.101	21.282	20.460
	5,250	3.907	–2.047	0.81	0.148	30.892	29.882
3 LA= *a* L^2 + *b*	4,350	0.091	0.814	0.81	0.383	79.005	77.056
	4,500	0.102	0.392	0.78	0.489	100.243	98.295
	4,650	0.101	0.560	0.79	0.297	56.137	54.067
	4,800	0.107	0.705	0.87	0.287	59.869	57.340
	4,950	0.148	0.683	0.57	0.218	44.969	43.630
	5,100	0.195	0.278	0.75	0.143	30.170	29.110
	5,250	0.151	0.716	0.79	0.163	34.432	32.861
4 LA = *a* W^2 + *b*	4,350	1.836	1.392	0.75	0.516	108.470	103.751
	4,500	1.777	1.369	0.79	0.474	100.678	95.181
	4,650	1.857	0.737	0.80	0.285	56.763	51.907
	4,800	2.050	0.720	0.82	0.411	86.931	82.149
	4,950	1.125	0.851	0.76	0.122	25.170	24.426
	5,100	1.335	0.629	0.83	0.097	20.556	19.672
	5,250	1.670	0.159	0.84	0.127	26.459	25.636
5 LA = *a* L^b	4,350	0.218	1.635	0.82	0.384	78.841	77.226
	4,500	0.178	1.738	0.76	0.504	103.342	101.342
	4,650	0.323	1.396	0.79	0.354	66.331	64.438
	4,800	0.278	1.554	0.87	0.292	59.696	58.354
	4,950	0.487	1.292	0.52	0.231	47.283	46.092
	5,100	0.336	1.638	0.75	0.152	31.816	30.912
	5,250	0.413	1.466	0.79	0.154	31.948	31.111
6 LA = *a* W^b	4,350	3.052	1.483	0.79	0.488	100.845	98.014
	4,500	3.084	1.416	0.85	0.414	85.580	83.209
	4,650	2.572	1.596	0.79	0.272	50.943	49.475
	4,800	2.704	1.712	0.85	0.393	81.287	78.494
	4,950	1.984	1.228	0.74	0.130	26.699	26.039
	5,100	1.951	1.506	0.81	0.096	20.106	19.521
	5,250	1.837	1.745	0.81	0.136	28.194	27.442
7 LA = *a* (L×W) + *b*	4,350	0.481	0.526	0.92	0.173	35.594	34.754
	4,500	0.498	0.468	0.92	0.170	36.122	34.259
	4,650	0.514	0.266	0.94	0.091	17.837	16.598
	4,800	0.532	0.363	0.95	0.112	23.720	22.295
	4,950	0.568	0.195	0.93	0.033	6.884	6.673
	5,100	0.619	0.098	0.95	0.026	5.540	5.361
	5,250	0.600	0.137	0.96	0.029	6.142	5.929

Low temperature at higher altitudes and to drought at lower altitudes, respectively. In arid environments, plants tend to develop relative narrow and long leaves (high L:W ratio), which is beneficial to prevent water loss through increasing sensible heat conduction and then reducing leaf transpiration (Parkhurs and Loucks 1972, Yates *et al.* 2010).

To summarize, model developed by two leaf dimensions (L and W, model 7) can estimate LA more accurately than those by single dimension (models 1–6). However, leaf shapes markedly vary with altitude, leading to significant differences in slopes and/or intercepts of model 7 between almost every two sites. Therefore, a common two-dimension model is unlikely to occur for *S. stoliczkai* across the whole altitudinal transect. The results hint that the varying leaf shape may alter the coefficient of LA estimation models. Researchers are suggested to take into account the effects of leaf shape variation when estimating LA by measuring L and W, especially when leaves were collected from different environments.

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