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Non-destructive leaf-area estimation for *Bergenia purpurascens* across timberline ecotone, southeast Tibet

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Accurate and non-destructive methods to determine individual leaf areas of plants are a useful tool in physiological and agronomic research. Determining an individual leaf area (LA) involves measurements of leaf length (L) and width (W), or some combinations of both parameters. Investigation was carried out in 2008 and 2009 to test whether a model could be developed to estimate leaf area of *Bergenia purpurascens* along an elevational gradient across a timberline ecotone, southeastern Tibet. A total of 786 leaves, 153–159 leaves for each 100-m elevation range, were measured in June 2008 for model construction. Coefficients of LA models incorporating both leaf dimensions (L and W) or W alone were independent of elevation, suggesting that a common model can be employed to estimate LA across the whole elevation range. A single-variable model using the leaf width ($LA = 1.44W^{1.90}$), which was simpler, more convenient and also allowed reliable LA estimations, was developed. By applying this equation to another independent dataset from a later experiment in June 2009 for model validation, we found that observed and predicted LAs exhibited a high degree of correlation ($R^2 = 0.95$). Therefore, this model can accurately estimate the leaf area of *B. purpurascens* across a timberline ecotone without use of any instruments.

Key words: *Bergenia purpurascens*, elevation, leaf area, leaf length and width, linear measurements, non-destructive methods, timberline ecotone, validation

Introduction

In terrestrial plants, leaves are the most important organs for carbon assimilation and gas exchange. Leaf area (LA) strongly influences light interception, and therefore plant growth and productivity, and becomes one of the key traits in ecophysiological and agronomic studies. However, accurate LA measurements of large numbers of leaves, especially in the field, is costly, time-consuming, laborious and usually destructive (Beerling & Fry 1990). Thus, for many fruit trees (Ramkhelawan & Bratwaite 1990, Potdar & Pawar 1991, Demirsoy & Demirsoy 2003, Demirsoy *et al*. 2004, 2005, Cittadini & Peri 2006, Serdar & Demirsoy 2006, Cristofori *et al*. 2007, 2008, Mendoza de-Gyves *et al*. 2007, Demirsoy 2009) and crop species (Rouphael *et al*. 2006, 2007, Peksen 2007, Rivera *et al*. 2007, Antunes *et al*. 2008, Tsialtas & Maslaris 2008, Fascella *et al*. 2009, Kandiannan *et al*. 2009, Kumar 2009, Zhou & Shoko 2009, Rouphael *et al*. 2010a, 2010b), non-destructive, easily applied models for LA estimation were developed based on simple measurements of leaf length and/or width. As an alternative to the LA measurement, this indirect, non-destructive method can provide accurate LA estimates and help *in situ* LA estimation.

Plant morphological traits, including leaf area, are determined by a combination of gene action and environmental effects. Many of the above-mentioned studies were concerned with the effects of different genotypes on LA estimation, but only a few paid attention to environmental effects (Rouphael *et al*. 2006, Serdar & Demirsoy 2006). To our knowledge, there are few published paper presenting altitudinal effects on LA estimates by using leaf dimensions (except the study by Mendoza de-Gyves *et al*. 2008), especially in high-altitude regions like a timberline ecotone above 4000 m a.s.l. A number of studies have found that an individual-leaf area in the same plant tends to decrease with increasing elevation (e.g., Geeske *et al*. 1994, Kao & Chang 2001), but we still do not know whether the varying size of leaf area alters the coefficients of models used to estimate leaf area from leaf dimensions, for leaf shape (ratio of leaf length to width) may change with leaf size in some species (*see* Rouphael *et al*. 2006).

Bergenia purpurascens, a perennial wintergreen herb belonging to Saxifragaceae, is widely distributed across the *Abies* timberline ecotone above 4000 m a.s.l. in the Sergyemla Mountains (one of the highest timberline positions in the world), southeast Tibet. Being a medicinal plant, its leaves and stems are widely used for relieving cough and reducing inflammation (Li *et al*. 2006). As is known, plants in high-altitude environment grow much slower than those in low-altitude regions because of the influence of low temperature (Körner 1999). In order to protect the limited natural resources, non-destructive method of LA estimation is of great necessity. Also, the timberline ecotone in southeast Tibet provides an ideal place for studying the relationship between plant and environment since this area suffers from litter disturbance. Therefore, our aims in this study are to test (1) whether altitudinal variation in leaf area affects coefficients of models used for LA estimation, and (2) whether there exists a general model that can simply, conveniently and accurately estimate LA from leaf length and/or width across the whole timberline ecotone.

Material and methods

Study sites

This study was conducted on a north-facing slope of a U-shaped valley (29°36´N, 94°36´E) near the peak of the Sergyemla Mountains in southeast Tibet, China. Along this slope (4150–4642 m a.s.l.), vegetation type changes from subalpine and timberline evergreen needle-leaved forests of *Abies georgei* var. *smithii* (< 4320 m a.s.l.) to alpine shrublands and/or grasslands (> 4320 m a.s.l.), forming a classical transition area called the timberline ecotone. According to the 3-year meteorological observations at 4390 m a.s.l., mean January and July air temperatures were –6.9 °C and 8.4 °C, respectively, and mean annual precipitation was 926.6 mm.

Sampling and leaf measurements

During early June of 2008, we set a straight line along the slope from about the foot (4150 m) towards the hilltop (4642 m). Along this line, the aboveground parts of three to six *Bergenia purpurascens* individuals were randomly sampled every 20 m, and the samples were immediately enclosed in plastic envelopes. Every 60 m along the slope, elevation was measured using GPS so that the elevation of each sample site could be calculated according to the linear relationship between measured elevation and slope distance (Elevation = 0.3805 × Distance + 4203, R^2 = 0.99, p < 0.001). In total, 786 leaves, 153–159 leaves for each elevation range (Table 1), were measured for predicting LA. Measurements (to the nearest millimeter) of maximum leaf length (L) (from lamina tip to the point of petiole intersection along the midrib) and width (W) (at the widest point perpendicular to the midrib) (Fig. 1) were carried out during the same day the leaves were detached. The actual one-side LA was determined with a portable area meter (CI-203, CID Inc., USA).

Model building and validation

Eight linear and power functions were deployed for LA estimation based on leaf length and/or width (Table 2). Selection of the best LA estimation equation was based on the following criteria: (1) combination of the highest coefficients of determination (R^2) and the lowest mean square errors (MSE); (2) simplicity and convenience of measurements (Rouphael *et al*. 2006, 2007). The latter is crucial at such high altitude as that of the timberline ecotone in southeast Tibet. Further, the difference in slopes and intercepts between models developed for each elevation range was tested using an analysis of covariance (ANCOVA). When no significant differences were found, data were pooled and a single relationship for LA prediction was created. Finally, according to the coefficients of determination between L and W across the elevation ranges (Table 1), we calculated the variance inflation factor (VIF, Marquardt 1970) and the tolerance value (T, Gill 1986) to detect collinearity in two-dimensional models (Eqs. 7 and 8 in Table 2). This is an important step before a model calibration, since applying two measurements (i.e. L and W) would introduce potential problems of collinearity, leading to poor precision in the estimates of corresponding regression coefficients.

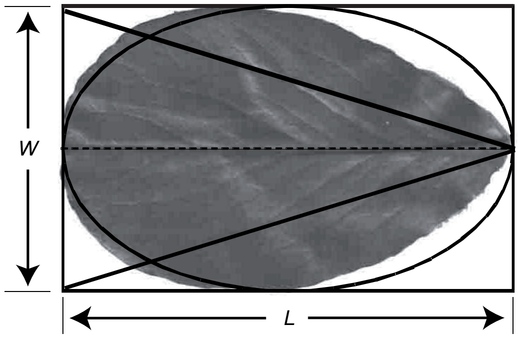

Fig. 1. Measurements of a *Bergenia purpurascens* leaf. The leaf area is between the area of a triangle and that of an ellipse.

In order to validate a selected model, a total of 168 leaves from 9 sites along the slope were further sampled (elevation ranged from 4180 m to 4600 m) in June 2009. Leaf area was predicted using the best one-dimensional model from the first experiment. The slope and intercept of the model were tested to see if they were significantly different from those of the 1:1 correspondence line. Regression and analysis of covariance (ANCOVA) analyses were performed using SPSS 13.0 package (SPSS Inc., Chicago, USA).

Results and discussion

In the dataset for model construction (n = 786), the leaf length, width and area (LA) ranges were 3.0–17.6 cm, 1.6–13.0 cm, and 3.5–172.9 cm^2, respectively. With increasing elevation, mean leaf length, width and LA all tended to decrease, whereas, leaf shape (L:W ratio) did not vary sig-

Table 1. *Bergenia purpurascens* leaf dimensions and leaf areas, ratio of length to width (L:W with standard error) along an elevational gradient. Coefficients of determination (R^2) and mean square errors (MSE) of the linear regression between leaf length and width are also given.

Elevation (m)	n	Leaf length (cm)			Leaf width (cm)			Leaf area (cm^2)			L:W (SE)	R^2	MSE
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max			
4150–4250	158	10.7	4.9	17.6	6.5	2.7	13.0	55.1	10.3	172.9	1.66(0.11)	0.83	2.45
4250–4350	158	9.4	3.0	16.5	5.9	1.6	10.2	44.9	3.5	121.3	1.61 (0.11)	0.88	2.11
4350–4450	153	8.9	3.5	14.1	5.6	2.3	9.7	39.4	6.4	101.9	1.60 (0.11)	0.80	2.31
4450–4550	158	9.0	3.1	14.6	5.5	2.1	9.8	38.7	4.8	104.9	1.63 (0.10)	0.83	2.07
4550–4640	159	8.0	3.7	12.5	4.9	2.4	8.4	30.6	7.1	71.1	1.64 (0.10)	0.82	1.73
Pooled	786	9.2	3.0	17.6	5.7	1.6	13.0	41.8	3.5	172.9	1.63 (0.11)	0.85	2.17

nificantly (Table 1).

For two-dimensional models, the degree of collinearity was analyzed. VIF and T ranged from 5.0 to 8.3 and 0.12 to 0.20, respectively. For each elevation range, VIF was < 10, and $T > 0.10$, suggesting that the collinearity between L and W can be considered negligible (Gill 1986) and both variables can be included.

Table 2. Intercepts (*a*) and constants (*b*) of the models estimating the *Bergenia purpurascens* leaf area (LA) from leaf length (*L*) and width (*W*).

Equation	Elevation (m)	*a*	*b*	R^2	MSE
1. LA = $aL + b$	4150–4250	10.98	–62.23	0.89	24.18
	4250–4350	8.64	–36.67	0.93	20.56
	4350–4450	8.08	–32.42	0.92	20.48
	4450–4550	7.93	–32.37	0.91	20.08
	4550–4640	6.73	–23.49	0.93	16.45
	Pooled	8.74	–38.74	0.90	18.24
2. LA = $aW + b$	4150–4250	15.02	–43.18	0.95	21.11
	4250–4350	13.83	–36.70	0.94	18.67
	4350–4450	12.35	–29.81	0.93	18.69
	4450–4550	13.10	–33.84	0.95	15.94
	4550–4640	11.76	–27.06	0.94	12.97
	Pooled	13.74	–36.53	0.94	16.80
3. LA = $aL^2 + b$	4150–4250	0.50	–4.85	0.93	32.77
	4250–4350	0.45	1.63	0.95	26.64
	4350–4450	0.44	2.11	0.93	24.37
	4450–4550	0.43	2.15	0.93	26.53
	4550–4640	0.42	2.22	0.94	23.36
	Pooled	0.46	0.17	0.94	16.50
4. LA= $aW^2 + b$	4150–4250	1.02	8.31	0.96	23.85
	4250–4350	1.15	1.51	0.97	21.98
	4350–4450	1.07	3.47	0.94	22.15
	4450–4550	1.11	2.65	0.96	20.43
	4550–4640	1.16	1.47	0.94	17.89
	Pooled	1.09	3.56	0.96	15.56
5. LA = aL^b	4150–4250	0.37	2.09	0.95	21.43
	4250–4350	0.52	1.95	0.97	18.52
	4350–4450	0.54	1.94	0.95	16.66
	4450–4550	0.60	1.87	0.95	26.53
	4550–4640	0.66	1.82	0.95	11.41
	Pooled	0.53	1.93	0.96	16.45
6. LA = aW^b	4150–4250	1.93	1.76	0.96	20.87
	4250–4350	1.36	1.93	0.97	17.38
	4350–4450	1.50	1.86	0.96	16.27
	4450–4550	1.39	1.91	0.96	20.43
	4550–4640	1.29	1.96	0.95	11.74
	Pooled	1.44	1.90	0.96	15.29
7. LA = $a(LW) + b$	4150–4250	0.75	–0.18	0.99	10.23
	4250–4350	0.75	0.09	0.99	8.50
	4350–4450	0.74	0.25	0.99	6.60
	4450–4550	0.73	0.53	0.99	8.43
	4550–4640	0.74	0.14	0.99	5.86
	Pooled	0.74	0.03	0.99	7.89
8. LA = $a(LW)^b$	4150–4250	0.76	0.99	0.99	10.36
	4250–4350	0.76	0.99	0.99	8.39
	4350–4450	0.77	0.99	0.99	6.53
	4450–4550	0.79	0.98	0.99	8.39
	4550–4640	0.78	0.99	0.99	5.76
	Pooled	0.76	0.99	0.99	7.91

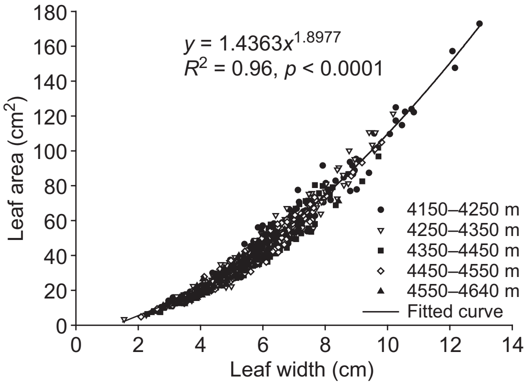

Fig. 2. Power function between observed values of leaf area and leaf width across different elevation ranges in the timberline ecotone. The trend line is for all pooled data in June 2008 ($n = 768$).

In each elevation range and across the whole transect, leaf length, width and functions of these dimensions were significantly correlated with LA for all eight equations (Table 2, $p < 0.0001$). Except for Eq. 1 at the lowest elevation range (4150–4250 m), all equations produced coefficients of determination (R^2) greater than 0.90 (Table 2). Among the eight models used for the LA estimation, the linear (Eq. 7 in Table 2) and power (Eq. 8 in Table 2) regressions including both leaf dimensions exhibited the highest R^2 (> 0.99, Table 2). Also, both models showed the smallest mean square errors (Table 2), indicating that they predict the best fits among the eight models. As far as the linear model of Eq. 7 (*see* Table 2) was concerned, the slope (i.e. leaf shape coefficient for pooled data) was 0.74, which agreed closely with the slopes calculated for leaves of fruit trees (0.66–0.74, *see* Cittadini & Peri 2006, 2007, Cristofori *et al*. 2008, Fallovo *et al*. 2008, Mendoza de-Gyves *et al*. 2008) or cultural crops (0.63–0.74, *see* Salerno *et al*. 2005, Rouphael *et al*. 2006, Peksen 2007, Rivera *et al*. 2007, Antunes *et al*. 2008, Fascella *et al*. 2009, Kandiannan *et al*. 2009). This coefficient can be described by a shape between an ellipse (0.78) and a triangle (0.5) of leaf length and width, because — as shown in Fig. 1 — actual leaf area of *B. purpurascens* is generally larger than a triangle but smaller than an ellipse.

Possible altitudinal differences were analyzed using the selected models. As far as models encompassing only leaf length were concerned, the slope of the models for different elevation ranges tended to decrease in linear functions (Eqs. 1 and 3, *see* Table 2) and increase in power functions (Eq. 5, *see* Table 2). However, the slopes of other models did not vary with elevation (*see* Table 2), and when leaf area estimations using an equation derived for a single elevation range *versus* the overall model were compared, they were not significantly different ($p > 0.05$) (*see* Table 2). These results suggest that leaf area estimation models incorporating leaf length and width or single leaf dimension of leaf width for *Bergenia purpurascens* are plausible.

If only one leaf dimension is used in LA estimation, W would be better than L, because models incorporating only W demonstrate higher R^2 and smaller mean square errors, and also their predictions were independent of elevation. Compared with the $L \times W$ models, single variable model incorporating W (Eq. 6 in Table 2) also allowed reliable LA estimations ($R^2 = 0.96$, MSE = 15.29) but at the expense of slight loss of accuracy. Given that it requires measurement of only one leaf dimension, which simplifies the measurement procedure, this model can be a good and non-destructive tool for studying dynamics of leaf growth, especially when measurement of a large number of leaves is needed (Williams & Martinson 2003). Therefore, data for the whole transect were pooled and a single power regression model ($LA = 1.44W^{1.90}$) was applied to predict LA (Fig. 2, $R^2 = 0.96$). Using this equation for the later validation experiment in June 2009, we compared the predicted LAs and observed LAs and found that they correlated well ($R^2 = 0.95$), and the linear regression for the relationship between observed and predicted values was not significantly different from the 1:1 line (Fig. 3). Moreover, the predicated values were close to the observed values, giving an underestimation of 1.2% in prediction. We preferred this power function encompassing only leaf width because of its simplicity and convenience, especially in such a cold area like timberline ecotone above 4000 m a.s.l. As stated by Rouphael *et al*. (2006, 2007), model selection requires a balance between predictive qualities of the model and the economy of including the least number of variables necessary to predict leaf area.

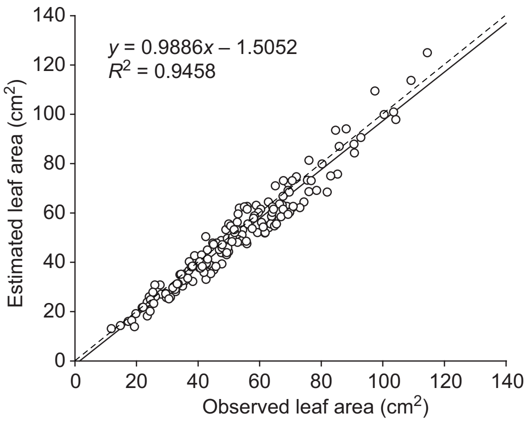

Fig. 3. Plot of predicted leaf area using modelled (LA = $1.44W^{1.90}$) *versus* observed values of leaf areas along an the elevation gradient from 4210 m to 4580 m during June 2009 (n = 168). Solid line represents the linear regression of Eq. 7 (*see* Table 2). Dashed line represents the 1:1 relationship between the predicted and observed values.

Conclusions

The power function encompassing only leaf width (Eq. 6 in Table 2) can provide accurate estimations of *Bergenia purpurascens* leaf area across different elevation ranges. Because leaf width can be easily measured in the field, this model would enable researchers to make non-destructive measurements or repeated measurements on the same leaves. Such model can accurately estimate leaf areas of large quantities of *B. purpurascens* leaves in many experimental conditions across the whole timberline ecotone without the use of any expensive instruments, e.g. a leaf area meter or digital camera with an image measurement software.

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