Simple Non-destructive Field Method to Estimate Leaf Area of a Rosette-leaved Perennial, *Ainsliaea apiculata*

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ロゼット植物キッコウハグマの葉面積の簡易推定法

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要旨：ロゼット植物では地際で葉が重なり合うため、採集せずに葉面積の測定することは困難である。そこで本研究では、個葉の画像解析によって面積とともに様々な部位のサイズを計測し、前者を非破壊で推定する推定式とその精度を検討した。供試材料には、キク科多年草キッコウハグマ（*Ainsliaea apiculata*）を用いた。同じ地域内（東京都御蔵島村御山山麓）の 7 個体について、葉面積推定のため、食害のない成葉を 1 個体 4～8 枚ずつ選び、それらの画像（400 dpi）をもとに、画像解析ソフトにより葉身の形質（個葉面積、葉身長、葉身幅、湾入部深、湾入部幅）と葉柄長を測定した。推定式として、個葉面積を被説明変数、葉の各部位のサイズを説明変数とする線形回帰および相対成長式を用いた。また、各個体の地上部の画像も撮影し、花茎長、葉数、すべての成葉の葉柄長を測定した。その結果、説明変数を 1 つから 4 つに増やすと決定係数 R^2 は 0.894 から 0.978 に向上できた。一方、葉柄長のみでも R^2＝0.884 が得られた。葉柄長による個葉面積の推定値を積算すると、個体の葉面積は 62.9±16.9 cm^2（変動係数 26.9%）となり、葉数と花茎長の実測値の変動係数（それぞれ 21.5%、32.7%）と比較して妥当と考えられた。

キーワード：ロゼット葉，キッコウハグマ，葉面積，推定，葉柄
Keywords: Rosette leaves, *Ainsliaea apiculata*, Leaf area, Estimation, Petiole

Introduction

Formation of rosette leaves is one of the strategies for perennial or biennial plants to survive a cold winter and to grow rapidly in early spring (Hayashi and Numata, 1967). Several species, e.g., dandelions (Genus *Taraxacum*) and plantains (Genus *Plantago*), have rosette leaves only. In horticultural cultivars of flowering plants, rosette formation is a serious problem for commercial value. Low temperature and dry conditions reportedly induce rosette formation in many cases, leading to abnormality or cessation of flower development. Thus, methods that prevent rosette formation, as well as the factors that induce it, have been studied from the viewpoint of growth environment and physiology (e.g., Fukuda *et al*., 1994; Takezaki *et al*., 2003; Yamashita and Imamura, 2007).

It is difficult to measure the area of rosette leaves without digging up the whole plant. This is because

Figure 1 *Ainsliaea apiculata*.
Photographed in Yamanashi Prefecture in October 2015.

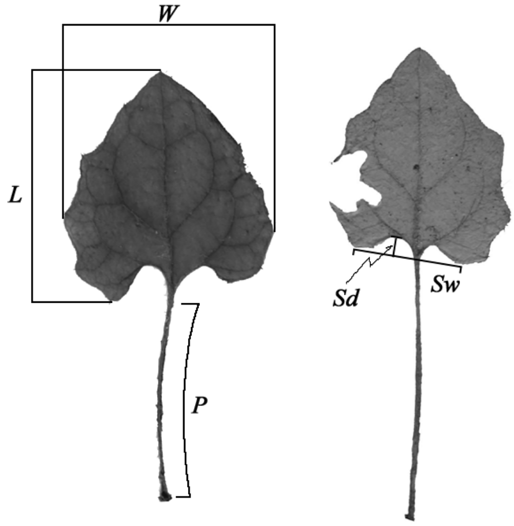

Figure 2 Leaf traits in *Ainsliaea apiculata*.
***L*: leaf lamina length, *W*: leaf lamina width, *Sd*: sinus depth, *Sw*: sinus width, and *P*: petiole length.**

they are radical leaves that foliate radially from the base: many leaves are crowded and lie next to one another, which makes it difficult to distinguish individual leaves by image analysis. This inconvenience prevents non-destructive measurements of growth of rosette plants, especially endangered species or plants in wildlife reserves where collection is prohibited. Therefore, a simple method to estimate leaf area is needed for the study of rosette-leaved plants.

We focused on *Ainsliaea apiculata* (Asteraceae) (Figure 1) as a model rosette-leaved plant. This species is distributed widely in Japan and produces small evergreen rosette leaves (Ohwi, 1992; Hori and Yokoi, 1999), which facilitates identifying its growth area and measurement. Since *A. apiculata* reportedly shows intra-specific morphological variation in leaf shape (Tsukaya *et al.*, 2007), a method to estimate leaf area will be of use to reveal further intra-specific variation.

In the present study, we measured the individual leaf area and also the sizes of various parts of the leaf as explanatory variables in *A. apiculata*. Based on our examination of the estimation accuracy, a simple method sufficient to estimate leaf area will be recommended.

Methods

We surveyed areas at the western foot of Mt. Oyama at elevations from 400 to 840 m of Mikura-jima Island of the Izu Islands, Japan, where *A. apiculata* is common.

In the summer of 2012, six sites where the species grew abundantly were surveyed. One or two normal ramets per site were selected, and four or more leaves per ramet were photographed (42 leaf images from 7 ramets in total). Based on the images of individual leaves at a resolution of 400 dpi (i.e., 0.0635 mm per dot), the traits of leaf lamina length (L), leaf lamina width (W), sinus depth (Sd), sinus width (Sw), petiole length (P) and individual leaf area were measured (Figure 2) with a Motic Images Plus 2.0S image-processing software (Speed Fair Co. Ltd., Hong Kong, China). For aboveground growth, length of flower stalk, number of leaves and petiole length of each leaf were also measured based on the images.

For the non-destructive estimation of individual leaf area, linear regression models were examined employing measurements of the traits of leaf lamina

Table 1 Accuracy of estimating individual leaf area (y) by leaf traits (x). A linear regression model, $y = a_0 + a_1 x_1 + a_2 x_2$, was applied.

Explanatory variables		Estimated coefficients			R^2	p
x_1	x_2	a_0	a_1	a_2	(n =42)	
L^2	-	0.967	0.505	-	0.894	<0.0001
L^2	Sd^2	1.058	0.469	2.484	0.901	<0.0001
W^2	-	0.079	0.802	-	0.942	<0.0001
W^2	Sw^2	0.030	0.856	-0.152	0.944	<0.0001
$L \times W$	-	0.254	0.679	-	0.977	<0.0001
$L \times W$	$Sd \times Sw$	0.237	0.688	-0.123	0.978	<0.0001

(L, W, Sd and Sw) and petiole (P) as explanatory variables. Linear models combining the traits in six ways (employing L^2; L^2 and Sd^2; W^2; W^2 and Sw^2; $L \times W$; $L \times W$ and $Sd \times Sw$) and an allometric estimation employing P were examined. The accuracy of the estimation was evaluated by the coefficient of determination (R^2).

Results

The accuracy of estimating the area of individual leaves by leaf lamina traits is demonstrated in Table 1. Estimation employing merely L^2 showed the lowest accuracy ($R^2 = 0.894$), while employing L^2 and Sd^2 showed slight improvement ($R^2 = 0.901$). However, these traits do not seem appropriate since the value of the estimated coefficient for a_2 (Sd^2) was positive (Table 1), but it should have a negative relationship to individual leaf area. Estimation employing merely W^2 showed higher accuracy ($R^2 = 0.942$), and employing W^2 and Sw^2 showed slight improvement ($R^2 = 0.944$). Estimation employing $L \times W$ showed even higher accuracy ($R^2 = 0.977$), and employing $L \times W$ and $Sd \times Sw$ showed slight improvement ($R^2 = 0.978$). The value of R^2 for each of the six types of estimation was significant (F-test, n = 42, p <0.0001). The last four types seem appropriate since the value of the estimated coefficient for a_2 (Sd^2, Sw^2 or $Sd \times Sw$) was negative (Table 1), but employing a_2 proved to have a small effect on improving the accuracy of estimation.

Estimation employing petiole length (P) is shown in Figure 3. Between individual leaf area (y) and P (x), a strong allometric relationship ($y = 1.632\ x^{1.073}$, $R^2 = 0.884$; F-test, p <0.0001) was observed, with an accuracy as high as the abovementioned estimation employing L^2.

Since individual leaf area could be approximately estimated only by P, leaf area of every leaf was estimated using the petiole length from the data for aboveground growth. Then, by summing up the estimated individual leaf areas, we aimed to obtain leaf area per ramet.

Table 2 shows the aboveground growth of each ramet. The number of rosette leaves and length of flower stalk were measured directly, while leaf area was an estimation based on petiole length. Leaf area per ramet was estimated as 62.9±16.9 cm^2 (mean±SD). Coefficient of variation (CV;

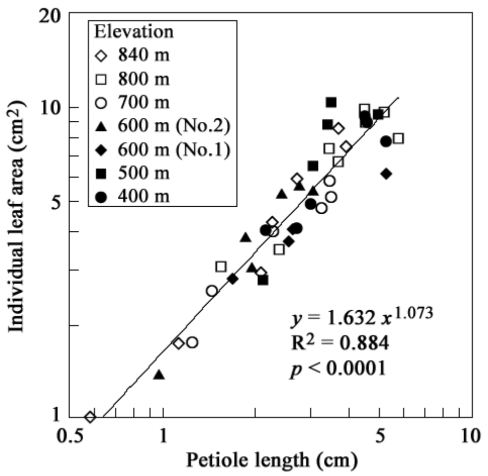

Figure 3 Allometric relationship between individual leaf area and petiole length in *Ainsliaea apiculata*.

Table 2 Aboveground growth of individual *Ainsliaea apiculata* ramets. Leaf area was estimated using the allometric relationship of Figure 3 between individual leaf area and petiole length.

Elevation (m)	Estimated leaf area (cm^2)	Number of rosette leaves	Length of flower stalk (cm)
400	76.8	19	9.4
500	67.8	17	17.0
600 (No.1)	71.7	17	18.6
600 (No.2)	41.9	15	9.3
700	59.6	23	12.4
800	83.0	14	22.7
840	39.1	12	16.3
Mean	62.9	16.7	15.1
± sd	16.9	3.6	4.9
CV (%)	26.9	21.5	32.7

SD/mean×100) of the leaf area was 26.9%, which was between the CV for the number of rosette leaves (21.5%) and for the length of flower stalk (32.7%).

Discussion

Our findings showed that individual leaf areas of *A. apiculata* can be estimated accurately by measuring leaf lamina traits. The more precisely the leaf size was measured (going from one to four traits), the more the accuracy of estimation improved (from R^2 = 0.894 to 0.978; Table 1). However, increasing the number of leaf traits to be measured is not feasible in field surveys: it requires more time and effort, and it is often impossible to measure all traits perfectly because of damage from herbivores or other factors. Many studies have used simple methods to estimate leaf area as a result of leaf damage (e.g., Trapp and Hendrix, 1988). For this reason, we favored the estimation employing only petiole length.

The petiole is a supporting structure of the leaf lamina, and therefore must be stable and persistent in response to various environmental factors. Petiole diameter is also reported to have a strong relationship to leaf size, probably to retain a sufficient number of conductive vessels (Yamada *et al*., 1999). In our field survey, we often observed intact petioles with the leaf lamina partially or wholly damaged. In other words, though not extremely accurately, petiole length will provide an estimate of intact individual leaf area, even after the leaf lamina has been worm-eaten or defoliated. Measurement of basal petiole diameter, in addition to petiole length if possible, is expected to improve the accuracy of leaf area estimation.

Leaf area per ramet was estimated to be 62.9±16.9 cm^2 with a CV of 26.9% (Table 2). This estimation takes into consideration partially worm-eaten lamina, because it was not estimated from leaf lamina traits; it might therefore be overestimated to some extent over actual leaf area. However, since other measurements of aboveground growth showed a similar CV (21.5% in the number of rosette leaves and 32.7% in the length of flower stalk), the estimation of leaf area per ramet is considered fairly appropriate.

However, it has been reported that growth environment alters petiole length and leaf area in some species (e.g. Huber *et al.*, 2008). Leaf shape is reported to require alterations to the coefficients of leaf area estimation models in *Saussurea stoliczkai* (Wang and Zhang, 2012). Although *A. apiculata* generally grows in the relatively uniform photo-environment of a shaded forest floor (Ohwi, 1992), intra-species geographic variation in its leaf shape has been reported (Tsukaya *et al*., 2007). Consequently, individual leaf areas and petiole lengths of several ramets should be measured in order to find an appropriate estimation equation for other survey areas.

Conclusions

In rosette leaves, it is difficult to measure the leaf area non-destructively. In the present study, we measured the individual leaf area, and also measured the sizes of various parts of the leaf as explanatory variables by image analysis, in order to find and examine estimation equations for individual leaf area in *A. apiculata* (Asteraceae).

Seven ramets from six elevations in the same region, Mikura-jima Island of the Izu Islands, Japan, were selected. Four or more intact leaves per ramet were photographed. Individual leaf area, leaf lamina traits (leaf lamina length (L), leaf lamina width (W), sinus depth (Sd), sinus width (Sw)) and petiole length (P)) were measured with image-processing software based on images at a resolution of 400 dpi. To assess aboveground growth, length of flower stalk, number of leaves, and petiole length of each leaf were also measured. For the estimation of individual leaf area, linear regression models employing the measurements of the leaf lamina and petiole traits were evaluated.

As a result, the more precisely the leaf size was measured (going from one to four traits), the more the accuracy of estimation improved (from R^2 = 0.894 to 0.978; p <0.0001). However, estimation employing only petiole length (P) showed an accuracy of $R^2 = 0.884$ ($y = 1.632\ x^{1.073}$, p <0.0001). Increasing the number of leaf traits to be measured is not feasible in field surveys, whereas petiole length has the advantage of being easy to measure even if the leaf lamina has been damaged.

Leaf area per ramet was estimated at 62.9±16.9 cm^2 with a CV of 26.9%, based on the petiole length. This estimation is considered fairly appropriate, since other measurements (number of rosette leaves and length of flower stalk) of aboveground growth showed similar values of CV.

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