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〈Short Communication〉

Comparison of Two Nondestructive Methods of Leaf Area Estimation

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ABSTRACT: We compared two nondestructive methods for leaf area estimation using leaves of 16 common plant species classified into six types depending on leaf shape. Relatively good linear relationships between actual leaf area (LA) and leaf length (L), width (W), or the product of length and width (LW) were found for ordinary leaves with lanceolate, oblanceolate, linear and sagitttate shapes with entire margins, serrate margins, mixed margins with a entire form and shallow lobes, and ordinary incised margins. LA was better correlated with LW than L or W, with R^2 > 0.91. However, for deeply incised lobes, LA estimation using LW showed low correlation coefficient values, indicating low accuracy. On the other hand, a method using photographic paper showed a good correlation between estimates of area based on the mass of a cut-out leaf image on a photographic sheet (PW) and actual leaf area for all types of leaf shape. Thus, the PW method for LA estimation can be applied to all shapes of leaf with high accuracy. The PW method takes a little more time and has a higher cost than leaf estimation methods using LW based on leaf dimensions. These results indicate that researchers should choose their nondestructive LA estimation method according to their research goals.

Key words: Leaf area, Leaf shape, Nondestructive estimation, Photographic paper

INTRODUCTION

Leaf area (LA) is one of the most useful parameters for understanding many aspects of functional plant performance, such as light and water use (Bunce 1989, Lott et al. 2000, Merilo et al. 2006, Tsialtas and Maslaris 2007). Leaf size is often linked to plant adaptations that determine the success or failure of a plant to settle in a given habitat (Meier and Leuschner 2008). In addition, leaf distribution in a community determines the productive structure of the plant community, which in turn determines the primary productivity in the community (VanWijk 2005). Thus, accurate assessment of leaf area is essential for the evaluation of plant performance on the individual, community and even ecosystem level, as leaf area will affect biomass production and nutrient cycling in the ecosystem (Meier and Leuschner 2008).

LA can be determined using instruments. However, this method is destructive and is not useful for field measurements in most cases. Portable scanners for LA measurement have recently been developed, but they are complex and expensive. The most common approach for nondestructive estimation of LA uses regression equations based on leaf dimensions such as midvein length and maximum width of the leaf. This method is simple, nondestructive and appropriate for research involving multiple measurements of LA on the same plant during plant growth. However, for plants with incised leaves or trifoliate leaves, leaf length and width may be poorly correlated, so this method may fail to estimate accurate LA. Consequently, various equations relating leaf dimensions to area have been studied on plant species and varieties such as cucumbers (Robbin and Pharr 1987), maize (Stewart and Dwyer 1999), sugar beets (Tsialtas and Maslaris 2005), sunflowers (Rouphael et al. 2007) and chestnuts (Serdar and Demirsory 2006) to derive methods for the accurate estimation of LA. Nomoto and Saeki (1969) developed another nondestructive method for LA estimation to trace the translocation of photosynthates in the field. However, little information about the accuracy and general applicability of this method is available. This nondestructive method for estimation of LA should be evaluated for leaves of various shapes, because sometimes continuous measurement of LA on the same leaves or plants during plant growth is essential for the clarification of plant ecophysiological processes. In addition, the method may be useful for ecophysiologists or agronomists who are looking for nondestructive, inexpensive, and reliable nondestructive LA estimation methods.

Our objectives were to evaluate two nondestructive methods for LA estimation by testing both methods on variously-shaped leaves from 16 plant species, and to determine the more reliable method for different types of research.

MATERIALS AND METHODS

Our investigation was carried out during the summers of 2006 and 2007.

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Plant Materials

We classified leaf shapes into six general patterns based on the set of leaf shapes observed in common plants, and then chose 16 plant species that met the criteria for the six leaf shape types. The representative leaf shapes are depicted in Fig. 1 and their characteristics are described in Table 1. Plant materials were obtained on the campus of Cheongju University, which is about 150 km away from Seoul, Korea, and from Mt. Wuam, which is behind the campus.

Methods

We obtained LA using three different methods. LA was estimated from a regression equation based on the midvein length of the leaf (L) and/or leaf width (W), estimated using the photographic paper method of Nomoto and Saeki (1969) with some modification, and then destructively measured with an instrument (AAM-8, Hayashi Denkou, Tokyo, Japan). In the field, each intact leaf, still attached to a stem or branch, was carefully placed on a sheet of photographic paper (Elieid, Ilford, No. 3, UK) and covered with a transparent acrylic plate, and then exposed to the sun for a few seconds, or a few minutes in cloudy weather, in order to capture the leaf's image. The photographic paper-leaf image was then sealed in a light-proof envelope and then the leaf was plucked. Each leaf was immediately sealed in a separate plastic bag, put in an iced chest, and transported to the laboratory for measurements of leaf dimensions and LA. Thirty leaves per species of different size were collected.

To establish the linear regression equations, the L and W of each leaf were measured with vernier calipers. For leaf types E, compound leaves, and F, trifoliate leaves, the maximum L and W were measured as if they were a single leaf. After measurement of L and W, the LA was destructively determined with the standard instrument.

For LA determination using photographic paper, we established a correlation equation from the relationship between the mass and area of the photographic paper in advance, and then used the same type of photographic paper for LA estimation throughout experiment. To estimate leaf area, the leaf's image that had been copied onto the photographic paper in the field was cut along the outline of the leaf image under dim light in the laboratory. The cut leaf image (PW) was then weighed with a digital balance, and LA was determined from the correlation equation between paper mass and area.

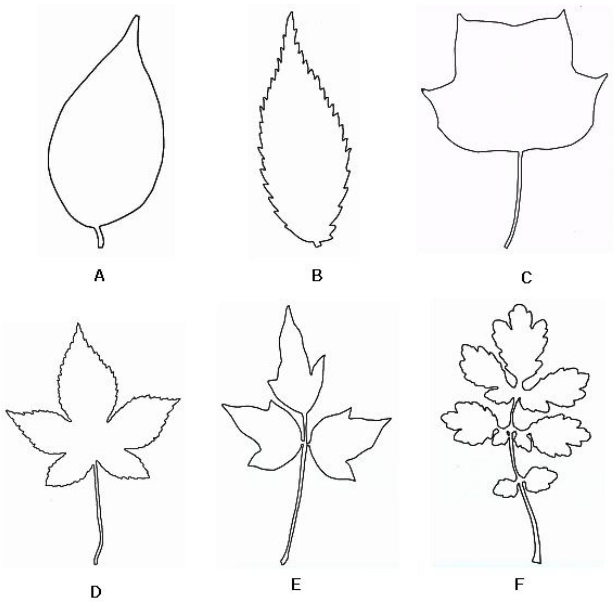

Fig. 1. Diagram showing six different types of leaves: A, entire leaf; B, dentate leaf; C, mixed form leaf with both entire and shallow margins; D, incised leaf; E, trifoliate leaf; and F, compound leaf or deeply lobed leaf.

RESULTS AND DISCUSSION

Although various regression equations have been used to estimate LA (Williams et al. 2003, Lu et al. 2004, Rouphael et al. 2007), we used a linear function for LA estimation because of its simplicity and applicability in field conditions. Regression analysis showed a relatively good linear relationship between LA and L, W, and the product of length and width (LW), irrespective of leaf type,

Table 1. Leaf types classified by leaf shape and characteristics

Type	Species	Characteristics
A	*Smilax china, Cornus officinalis, Achyranthes japonica*	Lanceolate shape, entire leaf
B	*Zelkova serrata, Quercus dentata, Betula platyphylla.* var. *japaonica*	Oblanceolate shape, dentate leaf
C	*Liriodendron tulipifera, Ginkgo biloba, Lindera obtusiloba* Blume var. *obtusiloba*	Mixed formed leaf of entire and shallow margin
D	*Humulus japonicus, Rubus crataegifolius, Acer pseudosieboldianum*	Incised leaf
E	*Dunbaria villosa, Poncirus trifoliata*	Trifoliate leaf
F	*Chelidonium majus* var. *asiaticum, Robinia pseudoacacia*	Compound leaf, deeply incised leaf

except for leaves of type F, with deeply incised lobes (Figs. 2, 3). However, the correlation coefficients were different for leaves with different dimensions. LA was more strongly correlated with LW than L or W for most leaves, with correlation coefficients greater than 0.91, while a value over 0.90 for L was shown only in *Zelkova serrata* (Type B; Fig. 2). A correlation coefficient greater than 0.90 for W was found for *Cornus officinalis*, *Liliodendron tulipifera* and *Humulus japonicus*. Thus, LW is shown to be a useful parameter for nondestructive estimation of leaf area in ordinary leaves with lanceolate, oblanceolate, linear and sagitttate shapes with entire mar- and ordinary incised margins (Type D, Fig. 1). However, LA was poorly correlated with LW in trifoliate leaves, and especially in gins

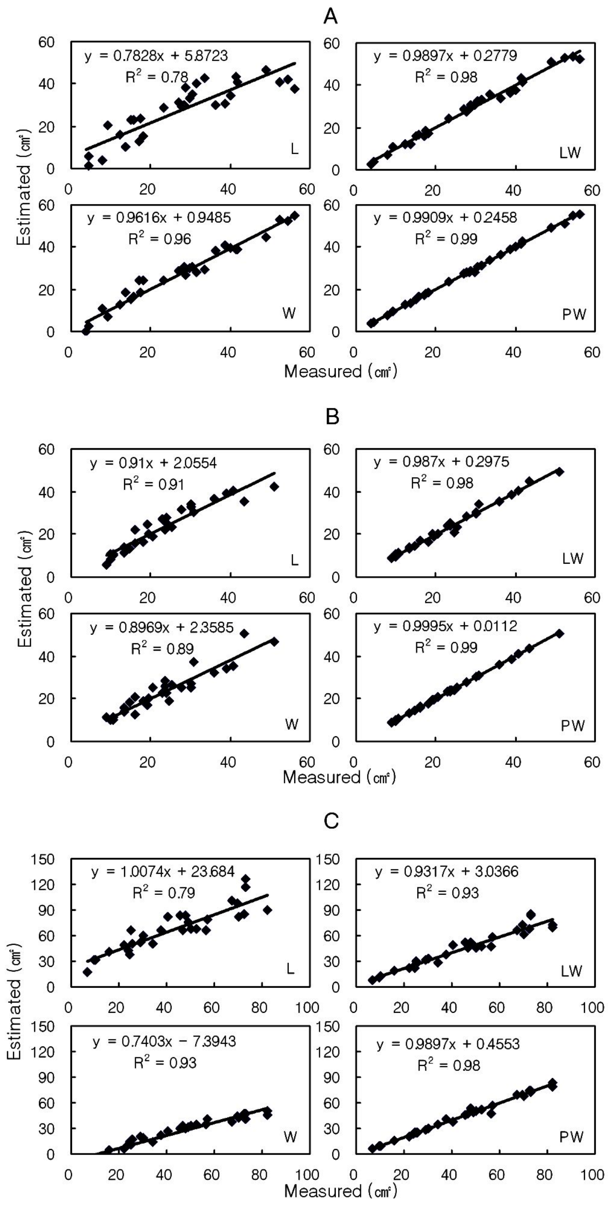

Fig. 2. Correlation between measured leaf area (Measured) and estimated leaf area (Estimated) from the relationship between leaf area (LA) and midvein of leaf (L), leaf width (W), the product of length and width (LW) and cut photographic paper weight with leaf image (PW) in representative plant species ($n = 30$ leaves per species). A, *Cornus officinalis*; B, *Zelkova serrata*; C, *Liliodendron tulipifera*.

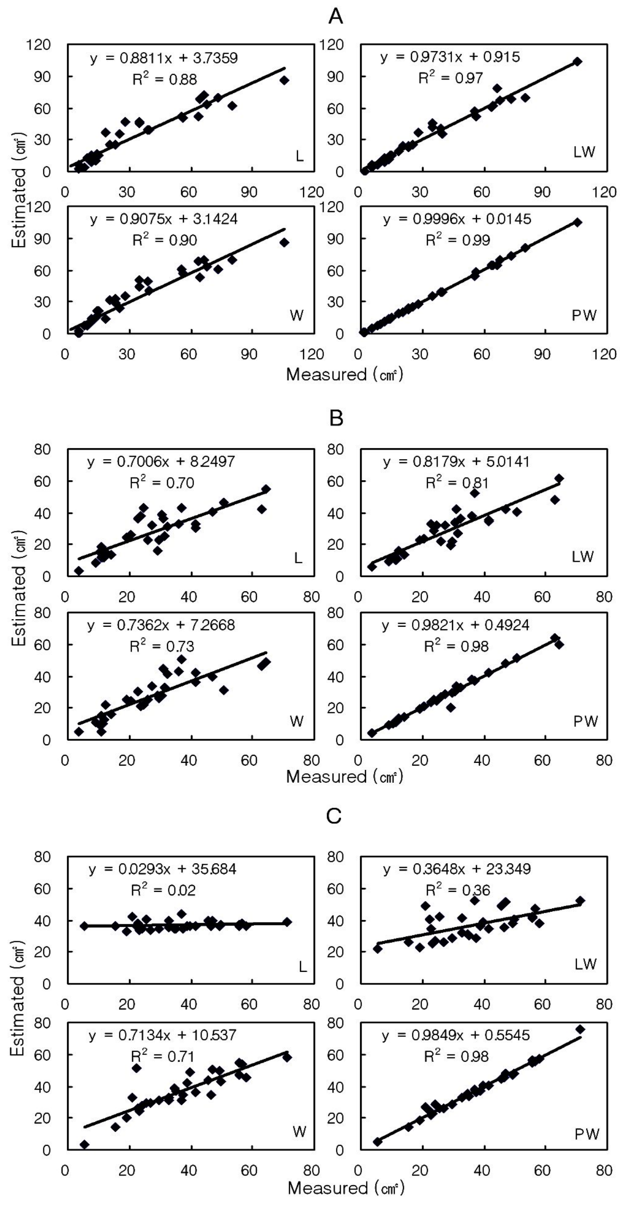

Fig. 3. Correlation between measured leaf area (Measured) and estimated leaf area (Estimated) from the relationship between leaf area (LA) and midvein of leaf (L), leaf width (W), the product of length and width (LW) and cut photographic paper weight with leaf image (PW) in representative plant species ($n = 30$ leaves per species). A, *Humulus japonicus*; B, *Dunbaria villosa*; C, *Chelidonium majus* var. *asiaticum*.

(Type A), serrate margins (Type B) mixed margins (Type C) leaves with deeply incised lobes, such as *Chelidonium majus* var. *asiaticum* (Type F) for which the correlation coefficient was only 0.36. On the other hand, the results from LA estimation using photographic paper showed the best correlation with LA for all types of leaves (Figs. 2, 3). Thus, we established a representative set of correlation equations according to leaf type by plotting LA, LW and PW together (Fig. 4). We do not show graphs plotting LW vs. PW or LA in types A, B, C and D because they were highly correlated, with correlation coefficients greater than 0.98 for both methods. The difference in the correlations of LA with LW and PW are clear for leaves of types E and F, as shown in Fig. 4. This suggests that LA in any kind of leaves can be estimated nondestructively with high accuracy using the photographic paper method, while LA estimation methods using leaf dimensions such as L or W have limitation application for some leaf shapes. Consequently, the photographic paper method may be the best method for nondestructive LA estimation in the field, though the cost of photographic paper must be considered, and the method may take a little more time and effort compared with methods using LW to estimate LA. Thus, researchers should choose their method for leaf area estimation depending on their target species and their research goals. For some ecophysiologists or agronomists studying ecophysiological phenomena in plants, it is necessary to nondestructively estimate LA from the same leaves or plants at multiple times during plant growth. Both nondestructive estimation methods for LA would be useful for this type of study for most leaf types.

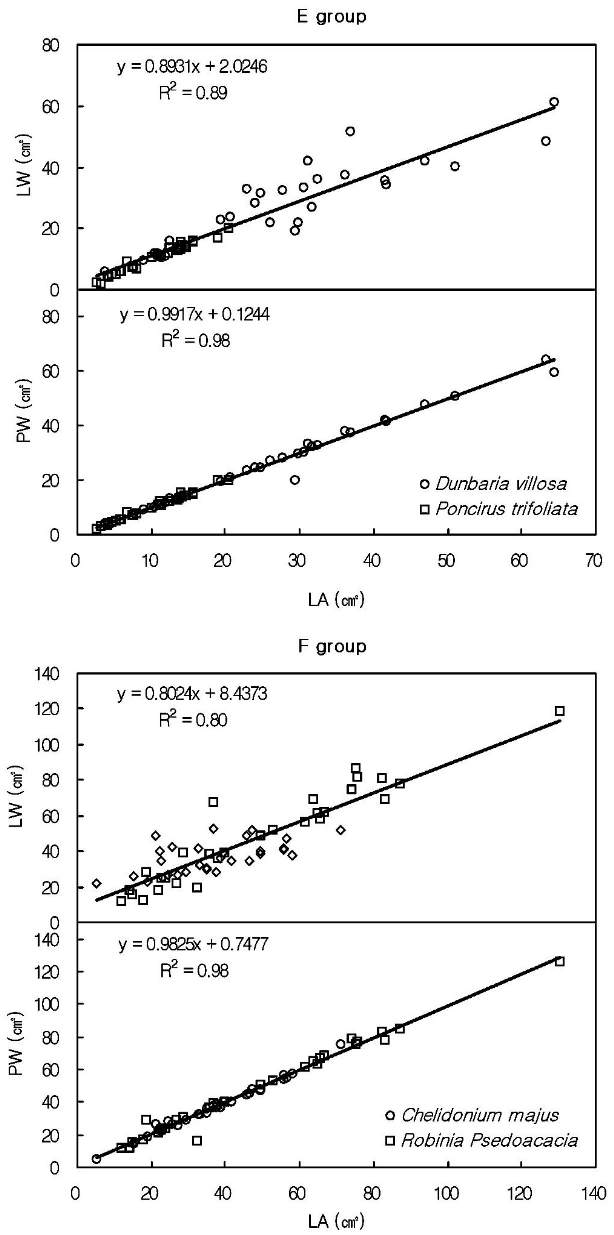

Fig. 4. Correlations between measured leaf area (LA), and the product of leaf length and width (LW: top) and the weight of cut photographic paper with leaf image (PW: bottom) in leaves of types E and F.

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