A Comparative Study of Methods for the Estimation of the Leaf Area in Forage Species

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Abstract. Estimating the leaf area of plant species entails many benefits, such as prediction of the productive potential and the achievement of optimal management practices in irrigation, fertilization and soil use. The purpose of this study was to compare the accuracy of four methods that are commonly employed to estimate leaf area in forage species. Three of these methods are categorized as destructive and include the estimation of the leaf area using a fixed device in the laboratory (Delta-meter) and two scan software packages (Laforem and Image Tool). Leaves are scanned and data were introduced into computer for surface analysis. The fourth method is a non-destructive one, which means that leaves were not harvested and the leaf area was estimated using a portable device (Li-3100) in the field. The results indicate that Li-3100 is very accurate for species with larger leaves, while destructive methods are necessary for species with smaller leaf area (<10cm^2).

Keywords: Destructive and non-destructive methods, scanning software, portable devices, regression analysis, correlation.

1 Introduction

The estimation of leaf area (LA) is necessary to assess the development and production potential of plant species (Kozlowski et al., 1991), thus the elaboration of methods enabling accurate and easy estimates has induced physiological and plant genetics research. Leaf area is directly linked to the photosynthetic efficiency of plant communities and determines the level of carbohydrates and the accumulation of dry matter (Williams 1987, Centritto et al., 2000, Caliskan et al. 2010). Also, there are important ecological implications that are connected with the estimation of the LA including the accurate knowledge of water and nutrient use as well as the plant soil-water relations and the proper implementation of managerial practices such as irrigation and fertilization (Sousa et al. 2005, Ugese et al. 2008).

The Leaf Area Index (LAI) (Dheebakaran and Jagannathan 2009) describes the magnitude of photosynthetic activity of a plant community and constitutes an

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important indicator describing the growth capacity – i.e. the yield of a crop - and development of plant species (Kvet et al., 1971, Caliskan et al. 2010). Knowledge of the variations of this indicator throughout the growth period constitutes a measure of plant productivity, as well as a means for understanding and monitoring ontogenetic changes and growth characteristics of plant species. The maximum value of the LAI is determined by the density of cultivation, regulated by the density of planting, the application of fertilizers and crop management operations. In natural ecosystems and plant communities, the LAI depends on water balance, nutrient availability, distribution of light within the crop canopy and other environmental factors (eg temperature). The LAI is the main factor determining the rate of biomass production of a crop (CGR) (Kvet et al, 1971) due to its significant impact of the net assimilation rate (NAR) (Watson, 1958).

The efficiency of methods estimating LA is determined by their level of precision, their time requirements, the availability of proper equipment and the experimental goals (Karatassiou et al., 2013). Leaf area estimation methods are generally classified as destructive and non-destructive. The former entail harvesting leaves from the plants and their examination using instruments or specialized scan software. A popular instrument is the area measurement system device (Delta – T Devices) that measures LA in integers (cm^2). Leaves are scanned and data are introduced to specialize computer software for surface analysis, of which Laforem (Lehsen**,** 2002) and Image tool (UTHSCSA 1996-2002) have been extensively employed. The latter methods (non-destructive) do not require leaves to be harvested from the plants and are based on statistical approaches (regression analysis) and optical techniques. The prediction of the leaf area using a non-destructive method is possible by applying the general relationship LA = b*L*W where in b is a coefficient, L the length of the leaf and W the width (Montgomery, 1911). This prediction equation is simple, accurate and brief and has been proven partially successful but only for specific leaf sizes. Pioneering applications of these methods have been reported by McKee (1964) and Montgomery (1911). Most recent studies focus mainly on estimation of LA of forest and agricultural crop and only few have attempted to estimate LA in other functional groups such as grass, legumes and shrub species (Karatassiou et al. 2013)

The purpose of this study was to compare the effectiveness of various methods for the estimation of the LA for several forage plant species. In particular, the statistical analysis sought to determine whether it is possible to make easy and accurate predictions by categorizing leaves by size, regardless the species. Furthermore, the methods were tested as to their accuracy for each species. Finally, using regression analysis linear equations were estimated enabling the prediction of the LA based on linear measurements of leaves (length and width).

2 Materials and methods

The research was conducted in natural vegetation in the farm of the Aristotle University of Thessaloniki, Northern Greece (longitude: 40°31’91’’, latitude: 23°59'58’’), at an altitude of 6m a.s.l. Measurements were taken in five forage species: *Cynodon dactylon* (L.) Pers (Grass), with leave size 0.62 – 2.27 cm^2, *Chrysopogon gryllus* (L.) Trin. (Grass) with leave size 2.00 – 9.71 cm^2, *Trifolium pratensis* L. (Legume) with leave size 2.00 – 35.34 cm^2, *Cercis siliguastrum* L. (Shrub) with leave size 17.00 – 72.67 cm^2 and *Anthemis arvensis* L. (Forb) with leave size 5.00 – 53.86 cm^2. Graph paper of various known dimensions was used (0.5- 50cm^2) to demonstrate the accuracy of the methods used to estimate the leaf area. Twelve plants of each species were randomly selected along a line. Two lines and a total of 24 plants were considered for each species (Cornelissen et al. 2003). From each plant two mature and intact fully expanded upper leaves without color deterioration and with same orientation were used. Initially the leaf area was measured in the field using the portable leaf area measurement system Li-3000A (LiCor Lincoln, Nebraska, USA). Then, the leaves were harvested and carried to the laboratory in a portable refrigerator. There, the fixed leaf area measurement device (Delta-T Devices Ltd, Cambridge, UK) was used to evaluate the LA of each species. In the following step both leaf and paper samples were scanned with the HP SCANJET 8250 scanner. Finally, the width (W) and length (L) of all leaves of each plant species were measured with a simple ruler.

Four methods for the estimation of the LAI of the five species were used and compared in this study.

A. Destructive methods.

A1. Fixed leaf area measurement device "Delta meter" (Delta-T Devices Ltd, Cambridge, UK). Delta meter is an electronic device designed and standardized under the Prom standard (ABB). The easiness of use constitutes one of the main advantages of the device, as only two buttons are enough to manage the whole process. The Delta meter measures the product LengthXWidth and shows the result on a Liquid Crystal Display (LCD) screen.

A2. Two different types of scan software

A2.1. Laforem (Lehsen, 2002) is software for image categorization especially designed for surveys regarding leaves and seeds. It uses data from conventional scanners to calculate the surface of leaves, thus being a cheap and user-friendly alternative. Care must be taken to choose the correct scale of analysis, in order to account for all the necessary characteristics of leaves; however, the application of this software could be proven complex and time-consuming for large leaves.

A2.2. Image tool (UTHSCSA 1996-2002) is an image processing and analysis software enabling illustration, analysis, compression, storage and printing of an image in grey scale. The software is compatible with other image processing packages and includes a built-in scripting language, which permits to automate tasks repeated frequently and to perform geometric transformations.

Non-destructive methods. The portable LA measurement device LI-3100 (LiCor-lincon Nebrasca USA) has been designed for biological and/or industrial applications. The samples are placed in celluloid between the drivers in the bottom surface of the portable device. Then the leaf is moved with a belt and the information is recorded at a frequency related to the speed of the belt. As the sheet moves between the drivers the image is reflected in a three-mirror system and the result is displayed on a screen (Li-Cor, LI-3100 Area Meter Instruction Manual, 1987).

The statistical analysis of the data included two parts. The first involved a correlation analysis (calculation of the r coefficient), in order to detect the method which best predicts the measured LA for smaller and larger leaves (at the 95% and 99% level). In addition, correlations were estimated between the results of each method. In the second step, a regression analysis was employed in order to formulate linear equations predicting the true LA for each one of the five species using only the linear measurements of leaves (lengthXwidth) as the dependent variable. Statistical analysis was performed using the SPSS statistical package (SPSS for Windows, standard version, release 21.0; SPSS, Inc., Chicago, USA).

3 Results and discussion

Table 1 presents the correlation coefficients between the estimated and the measured leaf area by average leaf size. It appears that all methods yield very satisfactory results. Non-destructive methods – the portable Li-Cor device - are particularly appropriate for larger leaf sizes (>10 cm, r = 0.9998). On the other hand, destructive methods seem to be slightly preferable to the use of Li-Cor for smaller leaves (<10 cm) - in particular the use of the Laforem scan software yields the most accurate results (r = 0.9974). In particular, Laforem is the most suitable for all the leaves of *Cynodon dactylon* and *Chrysopogon gryllus,* which are all under the threshold level of 10 cm^2, and the Li-Cor for all the leaves of *Cercis siliguastrum,* which are all larger than 10 cm^2. For the remaining two species the non-destructive method (Li-Cor) is more appropriate and also when leaf sizes are not categorized (r = 0.9997).

Table 1. Correlations between the measured and estimated leaf area (LA) by leaf size using various methods

Leaf size	r correlation coefficient			
	Destuctive methods			Non-destructive methods
	Image tool	Laforem	Delta meter	Li-Cor
≤10 cm^2	0.9872	**0.**9974	0.9962	0.9964
> 10 cm^2	0.9988	0.9977	0.9987	**0.9998**
0-55 cm	0.9983	0.9987	0.9990	**0.9997**

Table 2 presents the correlations between the LAs estimated for each forage species using all four alternative methods. The highest correlation is reported between the results of the two scan software methods (Image tool – Laforem). Nevertheless, it is also interesting to compare the results between the estimates of the use of the fixed device measurement system (Delta) with the remaining three. The results reveal higher correlations between the Delta and Image tool methods for *T. pretense* (LA 3- 34 cm^2) and *C. siliguastrum* (17 - 72cm^2) while the correlation between the Delta and Laforem methods is higher for the remaining three species, whose leaves are smaller on average *A. arvensis* (5 – 53 cm^2), *C. dactylon* (1 – 3 cm^2) and *C. gryllus (*4 – 10 cm^2). When it comes to predictions of the measured LA regardless of species, i.e. when the known area of graph paper was used, the highest correlation coefficient was estimated between the results of Delta meter and Li-Cor (r= 0,989, P<0.01).

Table 2. Correlations between the estimates of leaf area (LA) yielded by the four methods

Species	Image tool - Laforem	Image tool - Delta meter	Li-Cor - Delta meter	Laforem - Delta meter	Image tool - Li-Cor	Laforem-Li-Cor
Anthemis arvensis	**0.9998**	0.9922	0.9912	0.9932	0.9963	0.9961
Trifolium pretense	**0.9996**	*0.9731*	0.9712	0.9723	0.9994	0.9993
Cercis siliguastrum	**0.9996**	*0.9873*	0.9831	0.9872	0.9803	0.9802
Cynodon dactylon	**0.9497**	0.5322	0.1744[1]	0.6039	0.5914	0.6248
Chrysopogon gryllus	**0.9948**	0.7163	0.6293	0.7349	0.9338	0.9299

[*] All correlation coefficients are significant at the 99% level except for the one marked by ([1])

In Table 3 the results of five regression analysis models are reported, where the dependent variable is the LA estimated for the five species by the most appropriate method and the independent variable is the product Length X Width for the leaves of each species. Li-Cor produced the most satisfactory results for *A. arvensis* (R^2 = 0.8645) and *T. pretense* (R^2 = 0.9616). Image tool yielded the most reliable estimates for *C. siliguastrum* (R^2 = 0.9547) and *C. gryllus* (R^2 = 0.5435).The most suitable method for the estimation of the LA of *C. dactylon* was Laforem (R^2 = 0.7072).

Table 3. Linear equations of the leaf area (LA) for five forage species (regression results).

Species	**Method**	**Linear equation (X = leaf lengthXwidth)**	R^2
Anthemis arvensis	Licor	LA = - 3.0188 + 9.0778X	0.8645
Trifolium pretense	Licor	LA = 0.9212 + 2.2614X	0.9616
Cercis siliguastrum	Image tool	LA = 0.8874 + 1.0637X	0.9547
Cynodon dactylon	Laforem	LA = 0.6565 + 0.2982X	0.7072
Chrysopogon gryllus	Image tool	LA =3.2727 + 0.2641X	0.5435

4 Conclusions

The use of alternative methods for the estimation of the leaf area can lead to variable results. This study shows that it is relatively easy to categorize species according to their leaf size and to estimate their LA using uniform methodologies based only on their leaf size, rather than estimating species-specific linear equations. The use of a portable device (Li-Cor) in field contitions, which constitutes a non destructive method, is very suitable for species with larger leaves on average, while destructive methods are necessary for species with smaller average LA (<10cm^2).

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