Methods for estimating leaf area in forages species

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Abstract

Leaf area is an important variable for ecophysiological studies since it plays an important role in light interception, photosynthesis, water and nutrient use, crop growth and development. Moreover, understanding the properties of the leaf area could provide valuable information regarding cultural practices such as irrigation, fertilization, pruning etc. Nevertheless, determination of the leaf area is not an easy task, and there has been a great variety of methods developed. We present the most frequently used, direct and indirect techniques to estimate leaf area in forage species, and their advantages and disadvantages are discussed. Direct methods usually require removing leaves and then determining the leaf area; these methods are destructive and require adequate, potentially expensive, equipment. Indirect, non-destructive, methods are user friendly, less expensive, and can provide accurate leaf area estimation. The latter methods offer reliable and inexpensive alternatives in horticultural experiments and may be also used to determine the relationship between leaf area and plant growth rate. However, selection of the most appropriate method for leaf area estimation should be based on experimental goals and available equipment.

Key words: Non-destructive methods, optical techniques, portable leaf area meter, prediction models, planimeter

Introduction

Quantitative evaluation of vegetation abundance and distribution in grassland is an important tool to measure the productivity and health of both grazed and protected grasslands (He et al. 2007). Leaf area (LA) is an important component to determine light interception, photosynthesis, water and nutrient use, crop growth and development (Caliskan et al. 2010). Moreover, LA could provide information regarding plant growth analysis, plant soil–water relations, and the effects of different plant treatments such as irrigation, fertilization, pruning etc (Sousa et al. 2005, Ugese et al. 2008). Measuring LA is useful in analysing the plant canopy architecture and it also allows determination of the leaf area index (LAI) (Dheebakaran and Jagannathan 2009). Accurate methods to determine LA of plants can be valuable in physiological and agronomic research and ecosystem function modelling.

In the literature most studies focus mainly on estimation of LA of forest and agricultural crop and only few have attempted to estimate LA in other

plant types such as shrub and grass canopies (Caliskan et al. 2010, Gonsamo Gosa et al. 2007). Here we review studies dealing with the most frequently used techniques to estimate leaf area in forage species, and we discuss their advantages and disadvantages.

Methods for measuring and estimating leaf area

There are various methodological approaches to measure plant LA, both direct and indirect. Direct methods usually require removing leaves and then determining directly the LA using optical techniques, planimetry photography, digital photography etc (Caldas et al. 1992, Torri et al. 2009). Leaf area can be assessed directly by using the harvesting method. After leaf collection, LA can be calculated by means of either gravimetric or planimetric techniques (Daughtry 1990, Jonckheere et al. 2004b).

In the gravimetric or photogravimetric method photocopies of the leaves are used based on the weight of the paper cut out of the leaf tracing, compared to the weight of known areas on the same paper. The gravimetric method correlates dry weight of leaves and LA using predetermined green-leaf-area-to-dry-weight ratios (leaf mass per area, LMA). It provides an accurate measurement of the area, but is a laborious technique when applied to a large number of leaves (Caldas et al 1992, Li et al. 2008). Furthermore, attention must be paid to the large spatial and temporal variations in LMA of many species. The gravimetric method is convenient when LAI has to be estimated out of very large leaf samples (Jonckheere et al. 2004b).

The planimetric method is based on the principle of the correlation between the individual LA and the number of area units covered by that leaf in a horizontal level. There are different planimeter types in the market for this purpose e.g. the Li-3100 (Licor, Nebraska, USA) that provides apart from the leaf area, also leaf length and width. The planimeter is a less time consuming technique but the precision is limited especially for relatively small and rolling leaves of forage species. A second type of planimeter is the video image analysis system, consisting of a video camera, a frame digitiser, a monitor, and a computer with appropriate software to analyse the data (Caldas et al. 1992, Jonckheere et al. 2004b). Many researchers have developed related protocols using a common desktop scanner and public domain software to measure existing leaf area. Measuring LA with a desktop scanner requires two steps: (a) to create an image file and (b) to calculate the area presented by the image (O'Neal et al. 2002). These methods permit automatic calculation of LA, leaf number, length and width and the area lost from herbivores or diseases depending on the computer

programs used. Extremely small leaf areas less than 0.15cm^2 can be measured by using the high-resolution adjustment scanner. The method is useful for growth analysis photosynthesis measurements and studies of herbivory (Caldas et al. 1992).

Direct measurement of LA is usually time consuming and labour intensive and often destructive. Consequently, many researchers have looked for alternative indirect and less time consuming methods (Brenner et al. 1995, Rico-García et al. 2009, Mokhtarpour et al. 2010). In indirect methods, LA is derived from other (more easily determined) parameters. Two categories of non-destructive, indirect methods are often reported: the regression analysis (mathematical equations) and the optical techniques (Rico-García et al. 2009). Leaf area can be estimated by using mathematical equations, which only require simple measurements of the leaf length and width (Mokhtarpour et al. 2010). Many researchers have developed mathematical equations to estimate LA by measuring leaf length and leaf width and calculated different combinations of them (Cittadini and Peri 2006, Serdar and Demirsoy 2006). Since leaf development is strongly related with crop growth, knowing the change in leaf area may be useful for estimating crop growth (Caliskan et al. 2010). Mathematical equation for estimating LA reduces sampling effort and cost, and is likely to increase precision in cases where samples of small leaf size are difficult to handle (Dheebakaran and Jagannathan 2009). Such equations allow researchers to estimate LA in relation to other factors such as drought stress and insect damage (Williams and Martinson 2003).

Many researchers have tried using new equipment and tools such as hand scanners or laser optic apparatuses for estimating plant LA, but these are very expensive investments for basic and simple research (Cirak et al. 2005, Serdar and Demirsoy 2006). Portable leaf area meters such as the Li-3000C (LICOR, Lincoln, NE), CI-201 (Delta-T devices, Cambridge) AM300 (ADC Bioscientific Ltd) or the handheld laser leaf area meter (CID Bio Science) overestimate the leaf area of small size leaves of forage plants.

Another non-destructive method to estimate leaf area is the spray method. In order to apply the method a room plant spray bottle or other similar device and a light but rigid sheet of non- porous material are necessary. Compared to the most of the other methods the spray method is cheaper, precision is slightly lower, but the measuring times are similar. The spray method could apply to any leaves, which are nearly flat, regardless of their shape (Korva and Forbes 1997).

Discussion

There are only few comparative studies dealing with methods measuring LA for grassland vegetation, despite the broad use of such methods in ecological studies (He et al. 2007). Most methods used to estimate LA involve defoliation and suffer of being destructive and laborious (time consuming) for forage species. Moreover, destructive sampling is undesirable, especially in studies involving small plots or small number of plants. Also, these methods require expensive equipment and high level of technical competence for operation and maintenance (Ugese et al. 2008). The estimation of LA with a desktop scanner is inexpensive and accurate for the small leaves of the forage species, while the desktop scanner has advantages in certain experimental situations where a prefeeding measurement of the leaf is impossible or undesirable and small amounts of feeding occur (O'Neal et al. 2002).

Non-destructive estimation of leaf area offers researchers reliable and inexpensive alternatives in horticultural experiments. Non-destructive LA measurements are often desirable because using the same plant over time can reduce variability in experiments in contrast to destructive sampling. Additionally, it eliminates the need for expensive leaf area meters (Sezgin and Çelik 1999). Portable leaf area meters usually overestimate the LA of small size and rolling leaves of forage species and are also very expensive for basic and simple research (Caliskan et al. 2010).

The estimation of leaf area by mathematical equation or regression analysis is a useful tool when plants cannot be destroyed for direct methods. Leaf area models, which can estimate leaf area without damaging the plant, can provide several advantages in horticultural experiments. Moreover, these models enable researchers to measure leaf area on the same plant during the plant growth period, reducing experimental noise (Serdar and Demirsoy 2006) This allows day to day estimates of leaf area throughout the growing season on the same plants without using extensive field plots and/or labor intensive leaf area harvesting and sampling (de Jesus Jr et al. 2001). Disadvantages of regression analysis include a priory development of an equation for each plant species and even variety (Li et al. 2008, Rico-García et al. 2009). The spray method, although not expensive, cannot serve as an alternative for forage species since it applies only in flat leaves.

Conclusions

Direct methods to measure leaf area in forage species are the most precise but extremely time consuming. Non-destructive and mathematical

approaches of modelling can be very convenient and useful for plant growth estimation. There are instruments providing non-destructive and rapid but not accurate estimates of leaf area for forage species. However, selection of the most appropriate method for estimation of the leaf area should be based on experimental goals and available time and equipment.

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