Computers and Electronics in Agriculture 153 (2018) 278–284

Contents lists available at ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Original papers

Methods of image acquisition and software development for leaf area measurements in pastures

Adriano Rogério Bruno Tech[a,*], André Luis Céspedes da Silva[a], Luiz Antonio Meira[a], Marcelo Eduardo de Oliveira[b], Lilian Elgalise Techio Pereira[c]

[a] Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, Basic Sciences Department, Pirassununga, São Paulo, Brazil
[b] São Carlos School of Engineering (EESC), University of São Paulo, Center for Water Resources and Applied Ecology, São Carlos, São Paulo, Brazil
[c] Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, Animal Science Department, Pirassununga, São Paulo, Brazil

ARTICLE INFO

Keywords:
Automated image processing
Image capturing devices
Leaf area meter

ABSTRACT

The development of software for automated image processing, with particular emphasis on agricultural monitoring applications, has increased in the last years. Leaf area measurements are important for several crops and pastures, once the leaf area index is related with growth and photosynthesis rates, being an essential parameter of process-based models of vegetation. The processing algorithms implemented in most software for leaf area measurements currently available require the determination of leaf dimensions. Hence, the automated processing of samples with multiple leaves is still limited, frequently requiring manual pre-processing of the images. In order to develop a new software system aimed at processing images of samples composed of multiple leaves without any requirements of manual pre-processing of the images, the USP-Leaf software was developed. The software's performance was compared by using different devices for image acquisition: a semiprofessional digital camera (Sony, resolution of 12 megapixels); a mobile phone (Lenovo k5, resolution of 5 megapixels) and a desktop scanner (HP B20, 300 dpi corresponding to 3.508 × 2.480 pixels). The results were validated by comparing the values obtained with the standard method (electronic planimeter model Li-Cor 3100). The experiment was carried out at the Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, Pirassununga, SP, Brazil. The leaf samples were obtained from *Brachiaria decumbens* Stapf. cv. Basilisk pastures. A total of 20 samples comprising 15 leaves were collected, from which the images were acquired with each device. Edge detection, filtering and thresholding algorithms were applied to identify the leaf section of the image against the background. Considering the leaf area measured with the electronic planimeter, the relative error rate of the software's estimates was lower than 7%, being highest when the scanner was used and lower with the digital camera. Pearson's correlation coefficients were higher than 95%, regardless of the device used for image capturing, indicating that the software was able to provide accurate estimates of leaf area. The linear regression equation associated with the estimated leaf area using the mobile phone showed the highest values for the intercept and the higher standard error associated with this parameter (2.9 ± 5.69), despite showing a slope close to 1 (1.0 ± 0.07). The leaf area estimates were close to the standard method, showing that the software's performance was not affected by the device used for image acquisition.

1. Introduction

The leaf area index (LAI) is considered an essential parameter in process-based models of vegetation canopy, since it is a biophysical characteristic of vegetal communities directly related to the rates of atmospheric gas exchange and photosynthesis, light interception, biomass partitioning, and primary productivity (Kaur et al., 2014; Radzali et al., 2016). In pastures, the standard direct method for measuring the leaf area index require harvesting of above-ground biomass on a given area and a subsampling on a fresh or dry weight basis (Laca and Lemaire, 2000). These samples are composed of several leaves, which should represent the average condition of the canopy. This procedure is considered a standard method (Bradshaw et al., 2007), since LAI estimates from single plants are not able to provide accurate results, given both the vertically and the horizontally heterogeneous nature of the plant population (Laca and Lemaire, 2000; Sbrissia and Da Silva, 2008).

* Corresponding author at: Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, Basic Sciences Department, Duque de Caxias Norte Avenue, 225, Pirassununga, São Paulo, Brazil.
E-mail address: adriano.tech@usp.br (A.R.B. Tech).

https://doi.org/10.1016/j.compag.2018.08.025
Received 2 February 2018; Received in revised form 6 August 2018; Accepted 14 August 2018

After sampling in the field, the leaf area is measured by using a leaf area meter or a sampling grid, which, despite being a destructive method, provides more accurate measurements comparing to a non-destructive method (Kaur et al., 2014). However, such destructive methods are disadvantageous for being expensive, since they require specific equipment for that purpose. Besides, measuring leaf dimensions manually, in the case of using a sampling grid, is time consuming (O'Neal et al., 2002; Radzali et al., 2016). Moreover, as the number of leaves in a sample is high and the leaf area meter requires flat and turgid leaves to avoid unreliable measurements (Rico-García et al., 2009; Santos et al., 2014), underestimations of leaf area in pastures can be observed if the time between the cutting and the measurements is long, particularly in species with high rates of water losses after cutting, or when several samples need to be evaluated.

Alternative methods have been developed in the last years, including models based on the measurement of leaf length and width or their combinations to provide indirect LAI estimations (Maldaner et al., 2009; Sousa et al., 2015; Costa et al., 2016). They consist of less expensive and non-destructive options, but the models are normally species-specific, vary in terms of growth stages in a given species (Lopes et al., 2007; Maldaner et al., 2009) and require several *in situ* measurements to calibrate the models and obtain reliable results. These methods have neither been able to properly consider irregular leaf edges, commonly observed in some species (Lopes et al., 2007), particularly legumes and herbs (Toebe et al., 2014). Moreover, for some crops, the existing models based on leaf dimensions are not providing accurate LAI estimates for modern cultivars with different leaf traits, requiring new calibrations frequently (Richter et al., 2014).

The development of software for automated image processing, with particular emphasis on agricultural monitoring applications, has increased in the last years. Software based on digital processing of images with high resolution acquired from several devices, including mobile phones, cameras or desktop scanners, allows for fast, easy and automatic calculation of leaf area (Guerrero et al., 2012; Ferreira et al., 2017). On the other hand, some of the available free software has shown limitations in the processing step regarding the quality of the image captured, which must not be too bright or too dark as it could lead to error or failure in the leaf area measurement (Radzali et al., 2016). Additionally, Kaur et al. (2014) pointed out that some software using desktop scanners is not compatible with multiple scanner types and the leaf-area measurement accuracy varies according to the image resolution. Despite these constraints, digital image processing consists in a powerful tool that has largely replaced older methods (Easlon and Bloom, 2014).

The free software ImageJ (National Institutes of Health, public domain) is one of the most used in image processing and has been able to provide accurate leaf area estimates for a wide range of vegetal species with different leaf shapes and sizes (Guerrero et al., 2012; Easlon and Bloom, 2014). When measuring individual leaves, the software is able to provide reliable results regardless of the device utilized to capture the image (mobile phone, digital web camera, semi-professional digital camera or desktop scanners) (Guerrero et al., 2012; Santos et al., 2014). However, some previous steps are required before the results are provided, which includes manual processing of the images to set thresholds, conversion to grayscale image or binary image, and pre-processing to remove noise depending on the quality of the image (Park et al., 2012). Moreover, Ahmad et al. (2015) observed differences in the estimated leaf area of winter cereals from a manual method and two free software (Easy Leaf Area and ImageJ). Authors showed that the results of leaf area measured by ImageJ were more accurate, however, the values recorded were lower than leaf area from the manual method, and pointed out that darkness in image, image size and quality influenced the leaf area values.

Based on the need for a new tool capable of processing images of samples composed of multiple leaves, the software named USP-Leaf was developed. The framework of the software was designed so as to enable the user to obtain leaf area measurements without any requirements for manual pre-processing of the images. Thus, the aim of this study was to evaluate if the software is able to provide reliable leaf area measurements from images composed of multiple leaves. To test the software's performance, the leaf area measurements determined from images taken with a digital camera, a desktop scanner and a mobile phone obtained with the USP-Leaf software were compared with the values provided by the standard method (the leaf area meter Li-Cor 3100).

2. Materials and methods

2.1. Sampling procedure

The experiment was carried out at the Faculty of Animal Science and Food Engineering (FZEA) of the University of São Paulo (USP), Pirassununga, SP, Brazil (21°36′N, 47°15′W, 620 m a.s.l.). *Brachiaria decumbens* Stapf. cv. Basilisk pastures were stablished in 2012 on a Typic Eutrudox (USDA Soil Taxonomy), and the slope of the area is moderately undulating. The experimental area consisted of 18 plots of 80 m^2 (10 × 8 m), and each plot was subjected to a frequent cutting regime, simulating the rotational stocking method, from March 2015 to March 2017. For the purpose of this experiment and acquisition of the images, samples were collected at the pre-cutting stage in March 2017. The field samples were taken to the laboratory, and 20 experimental subsamples comprised of 15 leaves were prepared, from which the images were acquired. The criterion used to define the number of leaves in each sample was the processing area of the scanner, considering the use of an A4 sheet (21 cm × 29.7 cm) as background, and avoiding leaf overlapping. Based on that, the number of leaves per sample was standardized to all other devices. It is worth noting that in case a sample contains a number of leaves greater than that of the present experiment, the software will allow the processing of two, three or more images. The user needs to inform the number of images composing the same sample, and the software will sum the leaf area of each image, providing a final value. However, for the purpose of this experiment, it was defined that each sample would be composed of only one image.

2.2. Experimental treatments and image acquisition

The aim of this study was to develop a software that could process images acquired from low cost devices as alternatives to the Li-Cor 3100. For this purpose, the experimental treatments encompassed four methods for leaf area determination – three digital image processing methods from different devices, and one standard leaf area meter, as follows: (i) a semiprofessional digital camera (Sony, resolution of 12 megapixels); (ii) a mobile phone (Lenovo k5, resolution of 5 megapixels); (iii) desktop scanner (HP B20, 300 dpi corresponding to 3.508 × 2.480 pixels); and (iv) the standard method, using an electronic planimeter (leaf area meter Li-Cor 3100).

Before image acquisition, the leaf area of each sample was measured with the leaf area meter Li-Cor 3100. Subsequently, all the leaves of each sample were distributed on a white A4 sheet (21 cm × 29.7 cm). On the upper left corner of the sheet of paper, a black square of 1 cm × 1 cm (1 cm^2) was drawn with a permanent marker, which was used during the image processing as the standard known area to calibrate the software (Radzali et al., 2016). The digital camera was positioned on an inverted 'L' shape fixed support (Rico-García et al., 2009) at 23 cm of height from the base of the support, ensuring that all leaves were located within the area captured in the image. The same procedure was adopted for the images obtained with the mobile phone. With these two devices, the images were captured without using flashlight. The images were acquired in a well-lighted room. For the desktop scanner, the same white sheet of paper adopted to the previous devices was used as a background for image acquisition. Three photographs/images from each one of the 20 samples were taken with each device, and stored in a memory card.