Scientia Horticulturae 156 (2013) 113–120

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

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

Optical methods for the non-destructive estimation of leaf area index in kohlrabi and lettuce

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ARTICLE INFO

Article history:
Received 27 January 2012
Received in revised form 18 February 2013
Accepted 2 April 2013

Keywords:
Brassica oleracea var. gongylodes L.
Lactuca sativa var. capitata L.
Leaf area index
Plant canopy analyser
Gap fraction

ABSTRACT

The dimensionless leaf area index (LAI) is a fundamental crop characteristic. Since the direct measurement of LAI or leaf area is labour intensive and destructive, fast and reliable indirect methods have been devised to estimate LAI of different crops. The objective of this work was to test indirect methods for the non-destructive estimation of LAI in kohlrabi (*Brassica oleracea var. gongylodes* L.) and lettuce (*Lactuca sativa var. capitata* L.). Focusing on the gap fraction methodology, digital photographs and simultaneous radiation interception measurements were taken using a Li-Cor plant canopy analyser (LAI-2200) on 12 sampling dates from planting to harvest, with concurrent destructive estimations of the leaf area. Several geometric protocols of the LAI-2200 and inversion algorithms of the accompanying software were evaluated. Very good indirect-direct LAI relationships were obtained for kohlrabi ($R^2 > 0.97$, $n = 12$) and lettuce ($R^2 > 0.99$, $n = 9$) for the most suitable protocols and algorithms.

1. Introduction

The leaf area index (LAI) of horticultural crops is an important plant characteristic, related directly to canopy photosynthesis and transpiration (Baret et al., 2010). Accordingly, the efficiency of many cultural treatments can be estimated using crop LAI (Campillo et al., 2010). LAI is defined as one-sided leaf area per stand area (Breda, 2003). The leaf area is usually considered to be the normal projected area of one leaf side. More generally, however, when considering needle-like leaves, for example, the leaf area is the hemi-surface area (Chen and Black, 1992). If non-leafy plant organs contribute substantially to the total surface (e.g. surface area of stems and fruits), the term plant area index (PAI), expressed as square metre of plant area per square metre of ground, is more appropriate.

Both indirect and direct methods can be applied to measure LAI. Direct estimation of LAI involves measuring the leaf area using imaging devices. Such estimations can be performed destructively using harvested leaves or non-destructively using in situ growing leaves. Since direct methods are very labour- and time-intensive, and consequently expensive, several indirect methods have been developed to circumvent these drawbacks (Campillo et al., 2010). Indirect methods are usually based on optical principles. An overview of the different indirect methods can be found in Jonckheere et al. (2004). Direct methods enable greater precision; indirect methods are more cost efficient and enable time-repeated measurements to be made of identical plots and spatial patches. The greater uncertainty of optical methods is frequently caused by a lack of discrimination between leaves, stems, branches and fruit. For this reason, PAI is usually obtained in place of LAI (Li-Cor, 2011). Although a certain non-leafy contribution by kohlrabi tubers is accounted for, the therm "LAI" is used throughout this study. A further problem is the mutual covering of leaves in a non-random or clustered fashion. This lack of randomness – known as clumping – depends on the plant distribution over the ground area and the leaf distribution within the plants' envelope. Clumping frequently occurs in row crops. Previous investigations revealed that indirect methods yield lower LAI than direct estimates (Breda, 2003; Gordon et al., 1994; Rover and Koch, 1995; Welles and Norman, 1991), thought to be caused primarily by clumping. The morphology of a single plant (e.g. location, distribution and size of leaves) and of the canopy (e.g. distribution of plants) can affect indirect measurements. To obtain reliable results, indirect methods have to be validated for each horticultural crop under investigation.

In this work, indirect measurements of LAI from gap fraction inversions are discussed and compared with direct reference LAI values for kohlrabi (*Brassica oleracea var. gongylodes* L.) and lettuce (*Lactuca sativa var. capitata* L.). Gap fractions are obtained from digital RGB (red, green and blue) images with a soil background (i.e. the camera points towards the soil) and multi-angular light interception measurements using an LAI-2200 plant canopy analyser (LAI-2200, Li-Cor).

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http://dx.doi.org/10.1016/j.scienta.2013.04.003

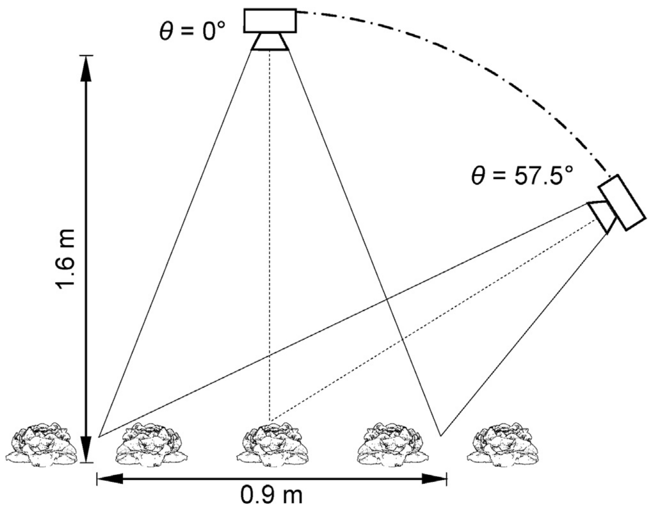

Fig. 1. Digital photography setup with zenith angles of $\theta = 0°$ and $\theta = 57.5°$ shown with lettuce.

2. Material and methods

At the field site of Leibniz-Institute for Vegetable and Ornamental Crops (Germany), kohlrabi (cv 'Lech' with a green tuber) and lettuce ('Torpedo') plants were set on 30 June 2011. The site is characterised by a sandy soil with 0.8% humus, 91% sand and 4.6% clay contents (Graefe et al., 2005). Both crops were grown in a double bed, with each bed comprising five rows. A regular planting grid of 0.3 m × 0.3 m (distance between rows × within the row) was adopted, resulting in a planting density of about 111,000 plants/ha. From 1 July to 16 August 2011, 15 plants were sampled twice a week from the inner three rows of one bed, then analysed. On two sampling dates, the weather conditions were unsuitable for sampling and taking optical measurements.

2.1. Leaf area measurements

All methods presented below where applied to the same 15 plants per date. Firstly, the non-destructive methods and afterwards the destructive one were performed. Measurements of LAI of the following date were accomplished by using 15 new plants. Digital photography provides the total area of green plantorgans (GAI). The plant canopy analyser measures PAI. As long as there are no senescent leaves, no significant differences occur between LAI, PAI and GAI in lettuce and (in case of green tubers) in kohlrabi.

2.1.1. Digital photography

Conventional digital RGB images were obtained using a Canon PowerShot G1 (10.5 Mpx); the optical axes were inclined at a nadir zenith angle $\theta = 0°$ (Campillo et al., 2008) and at 57.5° (Baret et al., 2010) (Fig. 1). Images were analysed using the software *CAN-EYE V6.1* (Weiss and Baret, 2010), which supports top-down viewing canopy images with soil background taken by conventional camera. Since the canopy is viewed from above, a crucial step is segmenting pixels representing soil and plants, which was achieved using the built-in classification algorithm in *CAN-EYE*. Potentially unsuitable image regions were masked out from further analysis. *CAN-EYE* uses focal length information from an EXIF header and a user-defined image sensor size to automatically vertical crop the image to ±5° of the target zenith angle (0° and 57.5°) in the vertical image domain. Following Baret et al. (2010), this was perpendicular to the row direction. The reason for choosing 57.5° is that the relationship between LAI and gap fraction (P_0) is independent of the leaf angle distribution for this particular zenith angle. The gap fraction is the fraction of soil pixels to the total number of pixels in the image (Baret et al., 2010), defined as follows:

$$P_0 = \exp\left(\frac{-G \times \text{LAI}}{\cos\theta}\right),$$

where G is the mean projection of unit foliage area. With the 57.5° zenith angle, the G-function is almost constantly 0.5 for all leaf angle distributions (Weiss et al., 2004). This fact can be used to simplify LAI computation. From a gap fraction inversion of segmented and cropped 57.5° images, *CAN-EYE* computes LAI directly via

$$\text{LAI} = -2 \times \ln(P_0) \times \cos(57.5°) = -\frac{\ln(P_0)}{0.93},$$

where P_0 is the gap fraction at the zenith angle 57.5° (Weiss, 2006). In contrast, nadir images primarily produce the crop cover fraction (*CCF*). Monsi and Saeki (1953) found the relationship

$$I = I_0 \times \exp(-K \times \text{LAI})$$

which connects CCF with LAI, where I is the shaded light intensity beneath the canopy, I_0 is the incident light intensity above the canopy and K is the extinction coefficient. CCF can be expressed as

$$\text{CCF} = 1 - \frac{I}{I_0}$$

and both equations can be combined and rearranged to

$$\text{LAI} = -\ln(1 - \text{CCF}),$$

implying an extinction coefficient of one.

2.1.2. Plant canopy analyser

The LAI-2200 plant canopy analyser (LAI-2200) is a dedicated device for indirectly measuring LAI (LICOR Bioscience USA, 2011). A fish eye-like optical lens estimates canopy gaps from radiation transmission measurements within five zenith angle ranges (centred angles: 7–68°). Transmission is calculated as the ratio between below canopy (B readings) and above canopy (A readings) readings of radiation intensity within a wavelength ranging from 320 to 490 nm. To reduce multiple reflections, wavelengths exceeding 490 nm are rejected. A detailed description of the technically very similar LAI-2000 can be found in Welles and Norman (1991).

LAI-2200 measurements were conducted in kohlrabi plots only. Since lettuce crops have a low plant height and highly clustered leaves, they were considered inappropriate. Following the LAI-2200 manual (LICOR Bioscience USA, 2011), three different protocols were tested throughout (Fig. 2). These protocols are similar to those employed by Boyd et al. (2002), Gordon et al. (1997), Grantz et al. (1993), and Hoffmann and Kluge-Severin (2010). Kohlrabi canopies have a row structure that is likely to clump. For this reason, only 90° and 45° view caps and diagonal transects were used, as recommended in the manual (Fig. 2).

Raw LAI-2200 readings were further processed offline using software *FV2200 1.2*, the LAI-2200 instrument software. This software offers diverse options for data processing and different inversion algorithms to calculate LAI. In this study, the following inversion algorithms were used:

- LAI: Default method, which accounts for the clumping factor (Li-Cor, 2011),
- CLS: Constrained least square method by Perry et al. (1988),
- Lang: Lang's method (Lang, 1987),
- Ellip: Least square method with ellipsoidal leaf angle distribution by Campbell (1986)

and