(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Remote Sensing of Environment 126 (2012) 240–247

Contents lists available at SciVerse ScienceDirect

Remote Sensing of Environment

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

How deep does a remote sensor sense? Expression of chlorophyll content in a maize canopy

Veronica S. Ciganda [a,1], Anatoly A. Gitelson [a,*], James Schepers [b]

[a] Center for Advanced Land Management Information Technology (CALMIT), School of Natural Resources, University of Nebraska-Lincoln, 303 Hardin Hall, 3310 Holdrege, Lincoln, NE 68583-0973, USA

[b] Agronomy and Horticulture Department (emeriti), University of Nebraska-Lincoln, 130 Keim Hall, Lincoln, NE 68583-0915, USA

ARTICLE INFO

Article history:
Received 25 August 2009
Received in revised form 7 August 2012
Accepted 11 August 2012
Available online xxxx

Keywords:
Remote sensing
Reflectance
Chlorophyll

ABSTRACT

Remote sensing offers a unique perspective of plant vigor based on reflectance of the crops' canopy. The goal of this study was to determine how deep into the maize canopy red-edge chlorophyll index, $CI_{red\ edge}$, was affected by foliar chlorophyll (Chl) content and leaf area. Reflectance in the range 400 to 900 nm was measured at both the leaf and canopy levels and was used to determine foliar Chl and total canopy Chl content using $CI_{red\ edge}$. Statistical techniques, a hierarchical regression and three Aikaike Information Criteria, were used to determine how many leaf layers are sensed by the $CI_{red\ edge}$. All statistical techniques showed that the $CI_{red\ edge}$ senses the chlorophyll content of the upper 7 to 9 leaf layers in a maize canopy and that remote sensing technique is able to accurately estimate maize canopy Chl content.

1. Introduction

The chlorophyll (Chl) content of a crop canopy is a biophysical variable that quantitatively expresses the photosynthetic capacity of vegetation. It is related to canopy biophysical parameters such as nitrogen content, above-ground biomass, green and total leaf area index, net ecosystem CO_2 exchange, and absorbed photosynthetically active radiation (PAR) (e.g., Evans, 1989; Gitelson et al., 2006). Therefore, it is not surprising that remote sensing has focused on the estimation of Chl content of the canopy to determine the vitality of vegetation and to detect vegetation stress (e.g., Barton, 2000; Gitelson et al., 2005; Le Maire et al., 2008; Ustin et al., 2009).

The relationship between light penetration and leaf area has been modeled using the Lambert–Beer law (e.g., Anderson, 1966). This relationship includes the light extinction coefficient defined as the fraction of light lost by scattering and absorption per unit distance within the canopy. The extinction coefficient is governed by canopy structure, which, in turn, depends on leaf angle distribution and canopy depth. Although these characteristics greatly affect the amount of reflected light, the extinction coefficient is assumed to be constant along the canopy profile. Such assumption does not consider that the vertical distribution of Chl content controls the total light reflected from the canopy, which is largely affected by the vertical distribution of leaf area. Moreover, there is important variability in the photosynthetic apparatus within the canopy that ranges from very dark green photosynthetically active leaves to pale green or senescent leaves (yellow to brown).

Tucker (1977) described the spectral reflectance of a grass canopy as an asymptotic curve where increments of vegetation density produce increments in spectral reflectance to the point where additional increase in leaf area index does not cause a change in reflectance. However, the question about canopy depth "sensed" by vegetation index remains unanswered. This problem is even more complex for a maize canopy with its bell-shaped vertical distribution of leaf area (Dwyer et al., 1992; Keating & Wafula, 1992; Valentinuz & Tollenaar, 2004) and Chl content (Ciganda et al., 2008). Vertical distribution of green LAI and Chl content in a maize canopy changes during the growing season (Ciganda et al., 2008). The vertical profile of the absorbed incoming PAR by a canopy with a parabolic distribution of leaves (similar to the maize leaf area distribution) has been modeled using a bell-shape function skewed to the upper part of the canopy (Kropff & Goudrian, 1994). It infers that the upper portion of the canopy is largely responsible for absorption of incoming light. However, it does not answer the question about how deep a sensor senses Chl content in a canopy with bell shaped Chl and leaf area distributions. In the case of maize, there is little, if any, information regarding how many leaf layers, from top to bottom, contribute to the canopy reflectance and to the estimation of canopy Chl content, derived from reflectance. In addition, the relative contribution of Chl content of each leaf layer to canopy reflectance through the growing season is not known.

Recently, a model for estimating foliar pigment content was developed (Gitelson et al., 2003) and then was used for accurate estimation of Chl content in crops at canopy level (Gitelson et al., 2005). The relationship between canopy Chl content, expressed as the product

* Corresponding author. Tel.: +1 402 4728386; fax: +1 402 4724608.
E-mail address: agitelson2@unl.edu (A.A. Gitelson).
[1] Now with INIA La Estanzuela, CC 39173 Colonia, Uruguay.

0034-4257/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.rse.2012.08.019

of chlorophyll content in the uppermost leaf and green leaf area, and red-edge chlorophyll index ($CI_{red\ edge}$) was established and used for estimating Chl content in maize and soybean canopies. Ciganda et al. (2009) showed that Chl content of collar leaf (uppermost leaf with a visible collar) and the ear leaf (leaf situated just under the ear), positioned in the middle of a maize canopy, relates closely to total canopy Chl content and further used $CI_{red\ edge}$ to accurately assess Chl content in maize.

The objective of this study is to determine how deep into the maize canopy the $CI_{red\ edge}$ senses and how accurately it estimates the canopy Chl content. We determined the number of leaf layers in a maize canopy, from top to bottom, which Chl content is sensed by $CI_{red\ edge}$ and, thus, affects the remote estimates of Chl content in the canopy. We also identified the relative contribution of Chl content in each leaf layer to the $CI_{red\ edge}$.

2. Materials and methods

2.1. Experimental setups

This study took advantage of an established research facility, which is part of the Carbon Sequestration Program at the University of Nebraska-Lincoln. The research facility consists of three agricultural fields of approximately 65 ha each, located in the vicinity of 41.175 N, 96.425 W. The cropping system was established in 2001 and differs among the three sites: site 1 was under continuous irrigated maize; site 2 is an irrigated maize-soybean rotation; and site 3 is a rainfed maize-soybean rotation. The study took place in the 2005 growing season. Sites 1 and 2 were planted with maize hybrids Dekalb 6375 (D-6375) and Pioneer brand 33B51 (P-33B51), respectively, at 85,000 seeds ha^{-1} with a row spacing of 76 cm. Site 3 was planted with maize hybrid Pioneer brand 31G68 (P-31G68) at 62,500 seeds ha^{-1} with a row spacing of 56 cm.

2.2. Canopy reflectance measurement

Canopy spectral measurements were taken twice weekly during the entire growing season of 2005 on each of the three sites. A dual-fiber system, with two inter-calibrated Ocean Optics USB2000 radiometers, mounted on an all-terrain sensor platform (Rundquist et al., 2004), was used to collect data in the range from 400 to 900 nm with a spectral resolution of about 1.5 nm. One of the radiometers, equipped with a 25° field-of-view optical fiber, was pointing downward to measure the upwelling radiance from the crop canopy (L_λ^{canopy}). The other radiometer, was pointing upward to simultaneously measure incident irradiance (E_λ^{inc}). Inter-calibration of the radiometers was accomplished by measuring the upwelling radiance (L_λ^{cal}) of a white Spectralon reflectance standard (Labsphere, Inc., North Sutton, NH), simultaneously with incident irradiance (E_λ^{cal}). Percent reflectance (ρ_λ) was calculated as:

$$\rho_\lambda = \left(L_\lambda^{canopy}/E_\lambda^{inc}\right) \times \left(E_\lambda^{cal}/L_\lambda^{cal}\right) \times 100\rho_\lambda^{cal} \quad (1)$$

where ρ_λ^{cal} is the reflectance of the Spectralon panel linearly interpolated to match the band centers of each radiometer.

Reflectance measurements were made within an area of *ca.* 0.8 ha for each of the three sites. During the growing season, the sensor was positioned at the same height *ca.* 4.8 m above the top of the canopy rendering an instantaneous field-of-view with a 2-m dia. A total of 36 spots within these areas was sampled per measurement date and site.

From each reflectance scan, the red-edge Chlorophyll Index ($CI_{red\ edge}$) was calculated as (Gitelson et al., 2005):

$$CI_{red\ edge} = \left(\rho_{NIR}/\rho_{red\ edge}\right) - 1 \quad (2)$$

where ρ_{NIR} is reflectance in the near infrared range from 770 through 800 nm and $\rho_{red\ edge}$ is the reflectance in the red-edge range from 720 to 730 nm. The mean value of the $CI_{red\ edge}$ of the 36 scans was computed to estimate Chl content in the canopy.

2.3. Plant sampling and labeling procedures

Three plants in each site were sampled on 15 dates (day of year; DOY 153 through DOY 263) from sites 1 and 3 and on 13 dates (DOY 166 through DOY 263) from site 2. The sampling period covered the period from early vegetative growing stages (three leaves) to the last reproductive stages when all kernels on the ear had attained their maximum dry weight or maximum dry matter accumulation. A total of 128 plants was sampled resulting in over 2000 leaves collected for further chlorophyll and leaf area measurements. On each sampling date, plants, considered representative of the growth stage for the entire site, were selected randomly from an area where remote canopy reflectance measurements were taken. Once the plants were selected, leaves were numerically labeled from top (leaf 1) to bottom positioned leaves using consecutive numbers. After labeling, the leaves were cut from the stem, placed in a sealed plastic bag, and brought to the laboratory inside a cooler.

2.4. Leaf reflectance and chlorophyll content

Leaf reflectance was measured in the spectral range from 400 to 900 nm using a leaf clip, with a 2.3-mm diam. bifurcated fiber-optic cable attached to both an Ocean Optics USB2000 spectroradiometer and to an Ocean Optics LS-1 tungsten halogen light source. The leaf clip allows individual leaves to be held at a 60° angle relative to the bifurcated fiber-optic. A Spectralon reflectance standard (99% reflectance) was scanned before each leaf measurement. Software called CDAP (CALMIT, University of Nebraska-Lincoln Data Management Program) was used to acquire and process the data from the sensor. The reflectance at each wavelength was calculated as the ratio of upwelling leaf radiance to the upwelling radiance of the Spectralon reflectance standard (for detail see Ciganda et al., 2009; Gitelson et al., 2003).

Each leaf was visually examined to identify and separate sections that were different in color. Leaf sections were marked, labeled and cut for further measurements. In the case of a leaf that was considered homogeneous in color, ten randomly distributed scans were made along the leaf margin (both sides of midrib). In the case of a leaf with a heterogeneous distribution of color, sections that appeared homogeneous in color were measured independently and ten randomly distributed scans were taken on each such leaf section. The mean of the reflectance was used to compute the $CI_{red\ edge}$.

Leaf Chl content (Chl_{leaf}), in mg Chl m^{-2}, per leaf area was estimated non-destructively from the mean of the reflectance obtained from each set of ten scans. We used a recently developed technique that relates $CI_{red\ edge}$ with Chl_{leaf} (Gitelson et al., 2003, 2006). This technique has been demonstrated to be robust and accurate in estimating leaf Chl content in maize (Ciganda et al., 2009). The linear relationship between analytically determined Chl_{leaf} and $CI_{red\ edge}$ for maize with a high coefficient of determination ($R^2 = 0.94$) has been established in the form (Ciganda et al., 2009):

$$Chl_{leaf}\left(mg\ m^{-2}\right) = 37.904 + 1353.7 \times CI_{red\ edge} \quad (3)$$

The algorithm (Eq. 3) was validated during growing season 2005 using independent data sets and showed the ability to accurately estimate Chl content ranged from 100 to more than 800 mg m^{-2} with root mean squared error (RMSE) below 37 mg m^{-2} (Ciganda et al., 2009). In each leaf, the Chl_{leaf} (in mg m^{-2}) and/or leaf section (Chl_{sect}) was estimated using Eq. (3) with $CI_{red\ edge}$ calculated by

Eq. (2) with reflectances averaged in the range from 720 to 730 nm (red-edge) and from 770 to 800 nm (NIR).

2.5. Canopy chlorophyll content

The total amount of Chl in each leaf (Chl_{leaf}^{total}), in grams of Chl, was calculated following a methodology developed by Ciganda et al. (2008). The area of each leaf, S_{leaf}, or the area of each leaf section, $S_{section}$ (in the case of leaves with sections of different greenness) was measured with a leaf area meter (Model LI-3100A, Li-Cor, Inc., Lincoln, NE). Total amount of Chl in each leaf was calculated as the product of leaf area, S_{leaf}, (in m^2) and its Chl content, Chl_{leaf} (in mg Chl m^{-2}) as follows:

$$Chl_{leaf}^{total} = Chl_{leaf} \times S_{leaf} \tag{4}$$

In the case of leaves with two or more sections of different greenness (i.e., "m" sections), total amount of Chl of the entire individual leaf was calculated as the sum of the products for each section using the following equation:

$$Chl_{leaf}^{total} = \sum_{i=1}^{m} Chl_{i\ \ section} \times S_{i\ \ section} \tag{5}$$

Total Chl content in the canopy (Chl_{canopy}) expressed as the amount of Chl per unit of ground area (i.e., g Chl m^{-2}), was calculated as the sum of the total amount of Chl in leaves of each plant, normalized by the ground area beneath one plant (S_g):

$$Chl_{canopy} = \sum_{i=1}^{n} Chl_{leaf}^{total} / S_g \tag{6}$$

Where n is number of leaves in each plant.

2.6. Statistical analysis

The relationship between $CI_{red\ edge}$, calculated from reflectance measured above the canopy, and total leaf Chl content, Chl_{leaf}^{total}, along the vertical profile of the canopy was analyzed using two approaches:

- hierarchical linear multiple regression,
- model selection using Aikake Information Criteria (AIC).

With the first approach, similarly to other multiple regression analysis, the hierarchical regression consists in establishing a set of independent variables that explain a proportion of the variance of the dependent variable. However, this analysis has the major advantage over other multiple regression methodologies (e.g., stepwise regression) in that the researcher determines the order of entry of the independent variables. The aforementioned attributes fit this study because the order of entering the leaf Chl content, from top to bottom, is essential to understand the effect of each leaf Chl content on the $CI_{red\ edge}$.

In this study, the dependent response variable (Y), computed across sites from the canopy reflectance data, was the $CI_{red\ edge}$ calculated using Eq. (2). The predictors or explanatory variables were the leaf Chl content (Chl_i) for each leaf positioned along the vertical profile of the maize canopy from top to bottom. Thus, Chl_i was entered in the model in a precise order: beginning from the uppermost leaf, followed by the leaf positioned immediately below that leaf, and going down through the canopy profile up to the 14th leaf (almost at the bottom of the canopy). Hence, the multiple regression took the form:

$$Y = b_{0i} + \sum_{i=1}^{n} b_i * Chl_i \tag{7}$$

Where the b_i are the regression coefficients, representing the amount the dependent variable Y changes with changes of the corresponding independent, Chl_i; b_{0i} is the intercept point where the regression line intercepts the y-axis, and it is different for each model; n is total number of leaves included in the model. The analysis ended up with a model with 14 parameters (each parameter corresponds to the Chl content in one leaf) not counting the intercept.

The models were evaluated using the adjusted coefficient of determination, R^2-adj. The R^2-adj is the R^2 adjusted for the degrees of freedom and does not necessarily increase as the number of variables in the model increases since it penalizes for the number of parameters included in the model. The formula for the R^2-adj is:

$$R^2\text{−adj} = 1 - \left[\left(1 - R^2\right)(n-1)/(n-k-1)\right] \tag{8}$$

Where n is the leaf number and k is the number of parameters in the model not counting the intercept (i.e., the number of Chl_i). The R^2-adj and the coefficients, b_{0i} and b_i, of each linear model were obtained using the *lm* function of the *R* statistical software (Hornik, 2006).

With the second approach, 14 models defined for the hierarchical analysis were set as candidate models (Table 1). Each set of models was analyzed independently for each of the three sites. The AIC was used to determine the best model from each set of 14 models (one model for each dependent variable) that was selected for each site. The AIC has its roots in Kulback–Leibler information and statistical maximum likelihood (Anderson et al., 2001). The value for AIC is:

$$AIC = -2\ln\left(l\left(\hat{\theta}|\text{data}\right)\right) + 2k \tag{9}$$

Where $\ln\left(l\left(\hat{\theta}|\text{data}\right)\right)$ is the value of the maximized log-likelihood over the unknown parameters (θ,), given the data and the model, and k is the number of model parameters. To select among models with very close AIC values, the difference between model AIC and the model with minimum AIC was evaluated:

$$\Delta_i = AIC_i - \text{minAIC} \tag{10}$$

The larger the Δ_i, the smaller the likelihood of that model being the best model in the set of candidate models considered (Westphal et al., 2003). In addition, the Akaike weights, w_i, were used as an indicator of the strength of evidence for the i model,

$$w_i = \frac{\exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{r=1}^{R} \exp\left(-\frac{1}{2}\Delta_r\right)} \tag{11}$$

Table 1
Set of a priori candidate models with k number of parameters. The dependent variable, Y, corresponds to the red-edge Chlorophyll Index $CI_{red\ edge} = (R_{NIR}/R_{red\ edge}) - 1$. The independent variables, Chl_i, represent the value of the Chl content from the top leaf, Chl_1, to the bottom leaf, Chl_{14}. The coefficients a_i and b_i are the intercept and the regression coefficient for each variable, respectively.

Model	k	Model formula
1	2	$Y = a_1 + b_1{*}Chl_1$
2	3	$Y = a_2 + b_1{*}Chl_1 + b_2{*}Chl_2$
3	4	$Y = a_3 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3$
4	5	$Y = a_4 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + b_4{*}Chl_4$
5	6	$Y = a_5 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_5{*}Chl_5$
6	7	$Y = a_6 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_6{*}Chl_6$
7	8	$Y = a_7 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_7{*}Chl_7$
8	9	$Y = a_8 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_8{*}Chl_8$
9	10	$Y = a_9 + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_9{*}Chl_9$
10	11	$Y = a_{10} + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_{10}{*}Chl_{10}$
11	12	$Y = a_{11} + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_{11}{*}Chl_{11}$
12	13	$Y = a_{12} + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_{12}{*}Chl_{12}$
13	14	$Y = a_{13} + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_{13}{*}Chl_{13}$
14	15	$Y = a_{14} + b_1{*}Chl_1 + b_2{*}Chl_2 + b_3{*}Chl_3 + \ldots\ldots\ldots + b_{14}{*}Chl_{14}$

The w_i can be interpreted as the probability that model i is the best Kulback–Leibler model in the set of models being considered (Anderson et al., 2000). The AIC values for each model were obtained using the AIC function of the R statistical software (Hornik, 2006).

3. Results

3.1. Chlorophyll distribution in a maize canopy

To understand how deep a remote sensing technique should sense inside a maize canopy for accurate Chl content estimation, it is necessary to know the distribution of Chl content in the canopy. Following this approach, Chl content was plotted versus leaf number from the top of a maize canopy for three stages of crop development: green-up stage (June 18), beginning of reproductive stage (July 26) and very late reproductive stage when senescence became conspicuous (September 6). The vertical distribution of Chl content in maize was bell-shaped regardless of crop growth stage (Fig. 1, see details in Ciganda et al., 2008). While the shape of Chl content distribution was similar during the season, the magnitude of Chl content was minimal in the beginning of the season, increased with crop development and became maximal in the beginning of reproductive stage (Fig. 1B), and then declined toward senescence (Fig. 1C).

The position of the leaf with maximal Chl content changed during the season (Fig. 2). In the beginning of the season, maximal Chl content was in leaves 6 to 7 that were about 0.75 to 1.0 m from the top of the canopy. In the middle of the season, leaves with maximal Chl content were located much deeper inside the canopy, in leaves 9–10 at about 1.25 to 1.5 m from the top. Toward the end of the season, leaves with maximal Chl were located closer (about 0.5–0.6 m) to the top of canopy (leaves 4–5). Therefore, to reach leaves with maximal Chl content, remote sensing techniques should sense Chl in leaf layers located at the depth of at least 1.25 to 1.5 m from the top of canopy.

To understand how each leaf layer contributes to total canopy Chl content, the cumulative Chl content from top to bottom of the canopy was calculated (Fig. 3). The cumulative Chl content showed minimum values at the top of the canopy, progressively increased reaching maximum values, and then remained invariant. While the magnitude of cumulative Chl content in the vertical profile of maize canopies varied during the growing season, the shape of the function 'leaf number vs. cumulative Chl' was similar for all sites with different hybrids and management practices (irrigated vs. rainfed crops). During the very early vegetative stages (June 2–15, day of year, DOY = 153–166), no differences were found among sites regarding both the shape of cumulative Chl content distribution and magnitude of cumulative Chl content. After June 15 (DOY = 173), the maize in the irrigated sites (1 and 2) showed higher values of cumulative Chl content in the vertical profile. The earlier senescence of the bottom leaves in plants of the rainfed site 3 (since August 10, DOY = 222) accounts for the difference between Chl content in irrigated and rainfed sites even larger. At very late reproductive stages (after September 7, DOY = 250), when senescence became conspicuous in all sites, the differences among cumulative Chl content between sites became negligible.

Importantly, the cumulative Chl content vs. leaf number relationship showed strong saturation at certain leaf layer, thus, addition of Chl content in leaves above certain numbers (lower in the canopy) did not increase cumulative Chl. Leaf position, where cumulative Chl reached the maximal values and did not change further, varies during the season. In the beginning of the season (DOY = 150–160), maximal cumulative Chl content occurred in leaves 7 to 8 (about 1 m from the top of plant), which were located near the middle of the plant. In the middle of the season (DOY around 190–220), it occurred in leaves 13 to 15 (about 2–2.25 m from the top of plant) that were located far below the middle leaf. In late reproductive

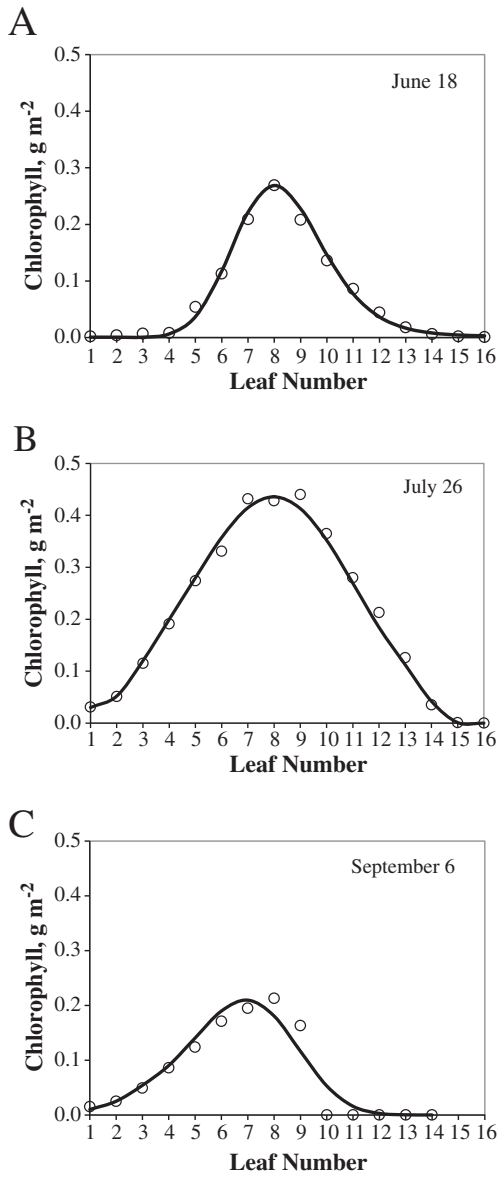

Fig. 1. Chlorophyll distribution in maize canopy. Leaf chlorophyll content versus leaf number for three phenological stages: (A) green up (day of year, DOY, 178); (B) beginning of reproductive stage (DOY 208), and (C) late reproductive stage, senescence (DOY 250). Top leaf corresponds to No. 1, ear leaf corresponds to No. 8.

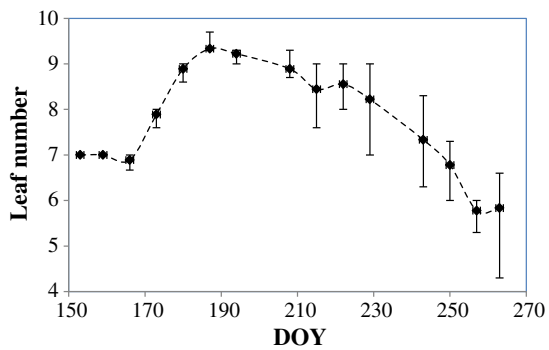

Fig. 2. Position of leaf with maximal chlorophyll content versus day of year, DOY. Top leaf corresponds to No. 1, ear leaf corresponds to No. 8. In the beginning of the season leaves 6–7 have maximal chlorophyll content. At time of maximal greenness, maximal chlorophyll content is in leaves 9 to 10 and in senescence leaves 4 to 5 are the greenest in the plant.

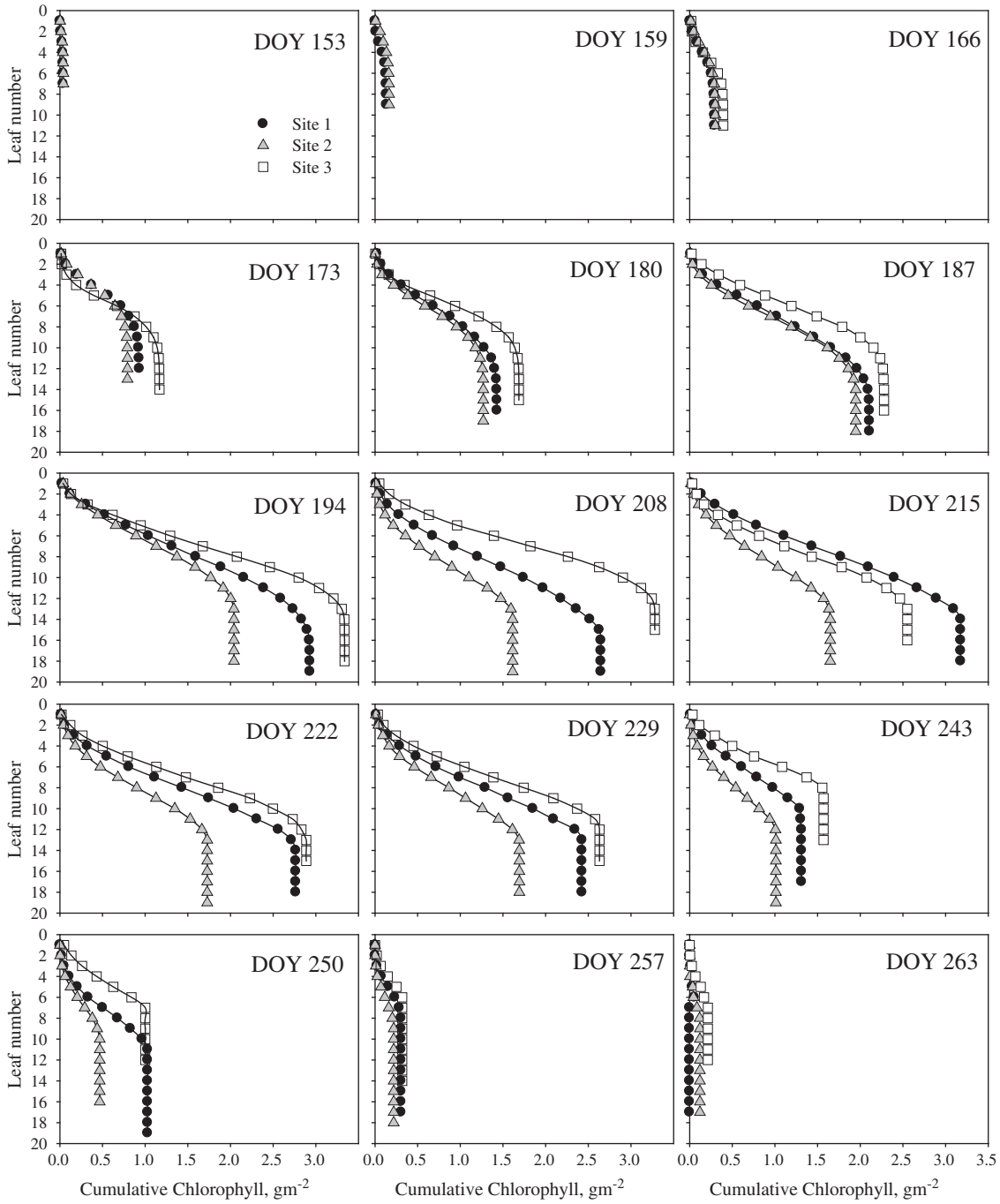

Fig. 3. Cumulative chlorophyll in the vertical profile at three maize sites for 15 sampling dates from June 2nd (DOY 153) through September 20th (DOY 263) of 2005. Cumulative Chl for each layer of the canopy was calculated as: $\mathrm{Chl}_i^{\mathrm{cumulative}} = \sum_{i=1}^{n} \mathrm{Chl}_i^{\mathrm{leaf}}$ where C_i^{leaf} is chlorophyll content in the *i*th leaf. Top leaf corresponds to No. 1, ear leaf corresponds to No. 8.

stages, the position of leaf with maximal cumulative Chl content shifted toward the middle of the plant and occurred around leaf 7 (about 1 m below the top of canopy). At this stage, the leaves positioned at the bottom of the canopy contributed minimally to the cumulative Chl. Thus, to reach the depth where cumulative Chl content becomes maximal, remote sensing techniques should sense Chl content in leaf layers located about 2–2.25 m below the top of the canopy.

However, slope of cumulative Chl content vs. leaf number decreased toward bottom leaf layers (Fig. 3), so few deep-located layers contribute less than nearby above layers. To determine number of leaves that contribute significantly to plant Chl content, we scaled cumulative Chl content, CumChl, as $(\mathrm{CumChl}_i - \mathrm{CumChl}_{\mathrm{min}})/(\mathrm{CumChl}_{\mathrm{max}} - \mathrm{CumChl}_{\mathrm{min}})$ and calculated number of leaves each having Chl content exceeding 5% of the maximal cumulative Chl, i.e., total canopy Chl content (Fig. 4). This number varied widely during the growing season. In the beginning and at the end of the season, only four to five top leaves located 0.5–0.6 m from the top of canopy significantly contributed to total plant Chl content. In the middle of the season, 11–12 leaves, located 1.5–1.7 m from the top of canopy, each having Chl values exceeding 5% of the total Chl content made significant contributions. These findings bring special requirements to remote techniques for estimating canopy Chl content that should be able to sense deep into the canopy.

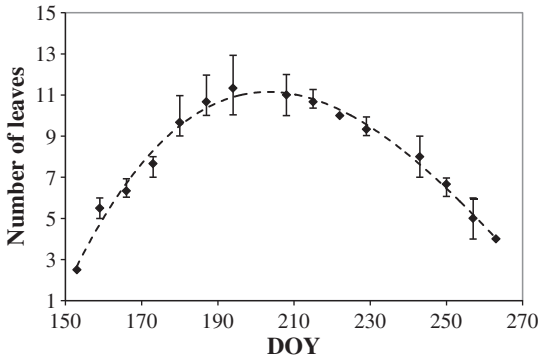

Fig. 4. Number of leaves with Chl content exceeding 5% of total canopy Chl content versus day of year (DOY).

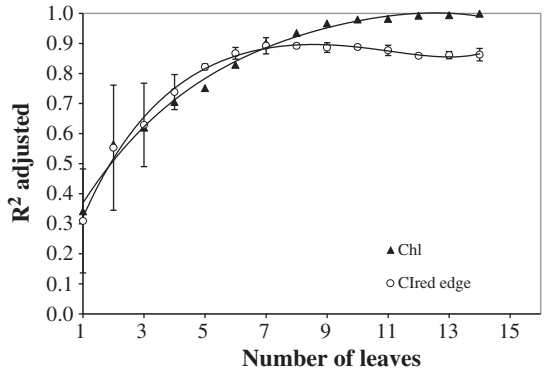

Fig. 5. Adjusted coefficient of determination for two hierarchical regressions: total canopy chlorophyll, $\mathrm{Chl} = b_{0i} + \sum_{i=1}^{n} b_i * \mathrm{Chl}_i$, and red-edge chlorophyll index, $\mathrm{CI}_{\mathrm{rededge}} = m_{0i} + \sum_{i=1}^{n} n_i * \mathrm{Chl}_i$, versus number of leaves (i.e. parameters). R^2-adjusted for the relationship $\mathrm{CI}_{\mathrm{rededge}} = m_{0i} + \sum_{i=1}^{n} n_i * \mathrm{Chl}_i$ were averaged for three sites and maximal and minimal values are presented by bars.

Findings can be summarized as follows: In the middle of the growing season

1. Ear leaf (No. 8) had maximal Chl content; it was located around 1.25 m from the top of the canopy.
2. Leaf layers above the ear leaf contained 55% to 65% of total maize Chl content.
3. Cumulative Chl content was maximized in leaf layers located about 2–2.25 m below the top of canopy.
4. The upper 11 to 12 leaf layers, located 1.5–1.7 m below the top of canopy (each having Chl content exceeding 5% of total Chl content), contained 75% to 85% of cumulative Chl content and, thus, significantly contribute to total canopy Chl content.

3.2. Estimation of chlorophyll content in a maize canopy

It is important to assess the accuracy of total canopy Chl content estimation and understand whether the red-edge chlorophyll index, $\mathrm{CI}_{\mathrm{red\ edge}}$, is able to meet the above determined requirements. To address this question, two approaches were considered: hierarchical regression analysis and Akaike Information Criteria (AIC).

3.2.1. Hierarchical regression

Hierarchical regression was used to understand (1) how total canopy chlorophyll content ($\mathrm{Chl}_{\mathrm{canopy}}$) relates to leaf Chl content, and (2) how chlorophyll index $\mathrm{CI}_{\mathrm{red\ edge}}$ relates to leaf Chl content. To assess hierarchical regression between $\mathrm{Chl}_{\mathrm{canopy}}$ and leaf chlorophyll, the relationship between $\mathrm{Chl}_{\mathrm{canopy}}$ and leaf chlorophyll ($b_{\mathrm{oi}} + \sum b_i \times \mathrm{Chl}_{\mathrm{i}}$) was calculated. In this expression, i is the leaf number that varied from 1 to 14 and $\mathrm{Chl}_{\mathrm{i}}$ is the chlorophyll content in the ith leaf. We began to calculate with leaf number one ($i = 1$), the uppermost leaf, and then added Chl content in the second leaf and so on until the 14th leaf of the canopy.

Adjusted coefficient of determination (R^2-adj) for the $\mathrm{Chl}_{\mathrm{canopy}}$ vs. $b_{\mathrm{oi}} + \sum b_i \times \mathrm{Chl}_{\mathrm{i}}$ relationship (Fig. 5) increased steadily with adding the Chl content in leaves until the 8th leaf (R^2-adj = 0.934) and 9th leaf (R^2-adj = 0.966) were successively included. Thereafter, the slope of the relationship declined drastically; including Chl content in leaves 11 through 14 in the regression brought very slight (0.02) increase in R^2-adj.

To determine hierarchical regression between $\mathrm{CI}_{\mathrm{red\ edge}}$ and leaf chlorophyll, as in the previous case, the relationship between $\mathrm{CI}_{\mathrm{red\ edge}}$ and leaf chlorophyll ($n_{\mathrm{oi}} + \sum m_i \times \mathrm{Chl}_i$) was calculated. The R^2-adj value increased steadily with adding the Chl content in leaves from the top toward the middle of the canopy (Fig. 5). However, the relationships for the upper four leaves were weak; R^2-adj was below 0.6. As the Chl content of successive leaves was added, R^2-adj increased to maximal value 0.9 (for 7 and 8 leaves) and decreased thereafter. It indicates that $\mathrm{CI}_{\mathrm{red\ edge}}$ related closely to Chl content in the upper 7–8 leaf layers.

The $\mathrm{CI}_{\mathrm{red\ edge}}$ vs. $n_{\mathrm{oi}} + \sum m_i \times \mathrm{Chl}_{\mathrm{i}}$ relationships had slightly different maximal R^2-adj values for each site: in site 1 maximal R^2-adj was for leaves 7 and 8, in site 2 for leaves 9 and 10, and in site 3 for leaves 8 to 10. Fig. 5 provides the average R^2-adj values with error bars showing the standard deviation of R^2-adj.

3.2.2. Akaike information criteria

The second way to select the best model for each dependent variable was based on the three AIC:

(1) minimal AIC values,
(2) the differences between model AIC and the model with minimal AIC (ΔAIC),
(3) Akaike weights, w_i.

Minimal AIC values were reached as 10, 9, and 8 leaves were included in the analyses for sites 1, 2, and 3, respectively (Table 2).

To more accurately select the best model, the differences between model AIC and the model with minimal AIC, ΔAIC, were calculated. The minimal values of ΔAIC were also found for models 8 through 10 (Table 2).

The Akaike weights, w_i, were used as another criterion to identify the best model. The w_i had very well pronounced peaks, indicating the best model for each site (Fig. 6, Table 2): the number of leaves including in the model varied from 8 to 10, indicating that $\mathrm{CI}_{\mathrm{red\ edge}}$ sensed Chl content in at least 8 top leaf layers in the canopy.

Importantly, both approaches, hierarchical regression and all three Aikaike Information Criteria, provided the same estimates of

Table 2
Akaike Information Criteria for the 14 selected models of $\mathrm{CI}_{\mathrm{red\ edge}}$ vs. leaf chlorophyll content, accumulated in one to 14 layers of leaves, for three sites studied.

Model	k	Site 1			Site 2			Site 3		
		AICc	ΔAIC	AICw	AICc	ΔAIC	AICw	AICc	ΔAIC	AICw
1	2	164.6	87.6	0.00	128.0	59.1	0.00	145.4	93.4	0.00
2	3	137.6	60.6	0.00	106.4	37.5	0.00	136.1	84.2	0.00
3	4	138.1	61.2	0.00	99.5	30.6	0.00	126.6	74.6	0.00
4	5	130.3	53.3	0.00	90.1	21.1	0.00	107.0	55.0	0.00
5	6	122.2	45.2	0.00	78.5	9.6	0.00	84.0	32.0	0.00
6	7	109.1	32.2	0.00	76.9	7.9	0.01	64.6	12.6	0.00
7	8	98.9	22.0	0.00	74.4	5.5	0.03	53.5	1.5	0.21
8	9	95.1	18.2	0.00	69.6	0.6	0.31	**52.0**	**0.0**	**0.45**
9	10	78.7	1.7	0.15	**68.9**	**0.0**	**0.44**	53.0	1.1	0.26
10	11	**76.9**	**0.0**	**0.36**	72.0	3.1	0.09	55.6	3.7	0.07
11	12	81.2	4.2	0.04	72.2	3.3	0.08	59.6	7.6	0.01
12	13	79.2	2.3	0.11	77.1	8.2	0.01	65.8	13.9	0.00
13	14	80.2	3.3	0.07	74.7	5.8	0.02	66.9	15.0	0.00
14	15	78.6	1.6	0.16	188.0	119.0	0.00	70.5	18.5	0.00

top leaf layers (7 to 9) that should be included in the model to get the best total Chl content estimation using $CI_{red\ edge}$. Thus, it indicated that the $CI_{red\ edge}$ senses as deep as about 1.25 m from the top of maize canopy. Deep $CI_{red\ edge}$ sensing within the canopy allowed accurate estimation of total Chl content of a maize canopy, ranging from 0.04 to 3.3 g m^{-2}, with coefficient of determination $R^2 = 0.95$ and RMSE of less than 0.25 g m^{-2} (Fig. 7).

4. Discussion

The $CI_{red\ edge}$ was demonstrated to be an accurate proxy of crop Chl content (Ciganda et al., 2008, 2009; Gitelson et al., 2005; Peng et al., 2011). Findings in this paper (Figs. 5 and 7) also showed high ability of $CI_{red\ edge}$ to estimate Chl content in maize. All four statistical tests (Figs. 5, 6 and Table 2) showed that remote estimation of Chl content was very accurate if leaf layers from the top (No. 1) to around the ear leaf (No. 8), located around 1–1.25 m below top of canopy, were included in the models. Thus, $CI_{red\ edge}$ sensed as deep as eight leaf layers.

The deep sensing capability of the $CI_{red\ edge}$ is due to choice of spectral bands used in the index. Canopy reflectance in the long wave part of the red-edge (720–730 nm) is much higher and absorption is much lower than those in the red spectral region, which is usually employed in vegetation indices (Gitelson, 2011; Gitelson & Merzlyak, 1996). This is the reason for deep penetration of the light in the red-edge region of the spectrum inside the canopy.

However, Chl content in eight leaf layers accounted only for 55% to 65% of total Chl content (Fig. 8). To estimate Chl content accurately, $CI_{red\ edge}$ needs to sense Chl content much deeper, at least in 11 to 12 top leaf layers, containing around 75% to 85% of total Chl content and located 1.5–1.7 m below the top of canopy. So, while all four statistical tests showed that sensing about eight top leaf layers was enough to accurately estimate total maize Chl content, it seemingly contradicts the fact that these leaf layers contain less than 65% of total canopy Chl content. In other words, in accord with the vertical distribution of Chl content, remote sensing techniques need to meet very stringent requirements — sensing below at least 12 top leaf layers; but it appears adequate to sense about only eight top leaf layers for accurate Chl content estimation.

It is clear from these findings that the close $CI_{red\ edge}$ vs. Chl content relationship (Figs. 5 and 7) was not because the $CI_{red\ edge}$ senses light reflected by leaves containing maximal canopy Chl content (i.e., top 10–12 leaf layers containing more than 80–90% of total Chl content). Therefore, it becomes important to learn the reason for the accurate estimation of Chl content in a canopy using techniques that sense Chl content in not more than 8–9 top leaf layers.

Our hypothesis was that Chl content in a top 7–8 layers, which is sensed by $CI_{red\ edge}$, well represented total canopy Chl content. To test this hypothesis, coefficient of determination of the linear relationship between Chl content in ear leaf (No. 8) and leaves located

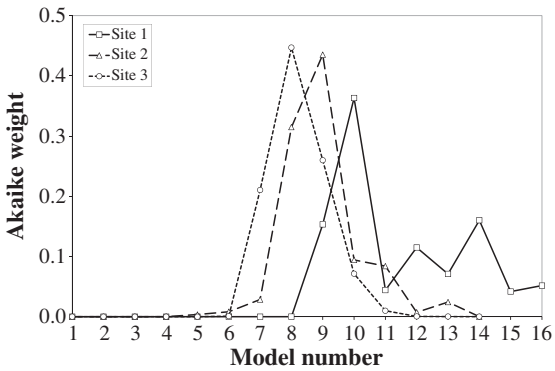

Fig. 6. Akaike weights versus model number for three sites.

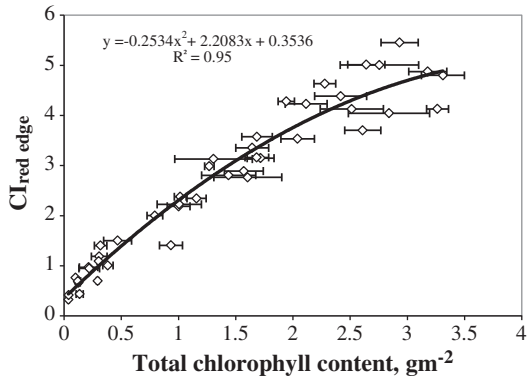

Fig. 7. Red-edge chlorophyll index, $CI_{red\ edge}$, versus average total chlorophyll content of three maize plants at three sites. Bars are minimal and maximal values of Chl content among three plants.

above and under ear leaf was calculated. As was expected, in the middle of the season, when green LAI/Chl content were maximal (DOY 208, July 26), there was a very close relationship between Chl content in the ear leaf and surrounding leaves (Fig. 9). The high correlation between Chl content in leaves located in the middle of canopy clearly documents why techniques sensing Chl content in about the 8 top leaf layers accurately estimates total canopy Chl content.

At growth stages when plants have more than 7–8 leaves, the reflectance of leaves positioned below the upper 8 leaves apparently contribute very little to the reflected light gathered by sensors. Importantly, the 7th and 8th leaves in maize have maximal Chl content, which closely relates to total canopy Chl (Figs. 1 and 4; see also Ciganda et al., 2008, 2009). Kropff and Goudrian (1994) showed that the middle leaves of a canopy (with a leaf area profile similar to a maize canopy) are responsible for the highest absorption of the incident PAR but do not contribute significantly to canopy reflection and, therefore, to R^2-adj of the $CI_{red\ edge}$ vs. $n_{oi} + \sum m_i \times Chl_i$ relationship. On the contrary, upper leaves, having less Chl content and being smaller, absorb less light but contribute significantly to canopy reflectance in the visible and red-edge regions and, thus, tend to increase R^2-adj.

However, as can be seen in Fig. 4, in the middle of growing season at least 12 top leaves significantly contribute to total Chl content in the canopy, while $CI_{red\ edge}$ senses Chl content in about 7 to 8 upper leaves. There is no contradiction between these findings because for

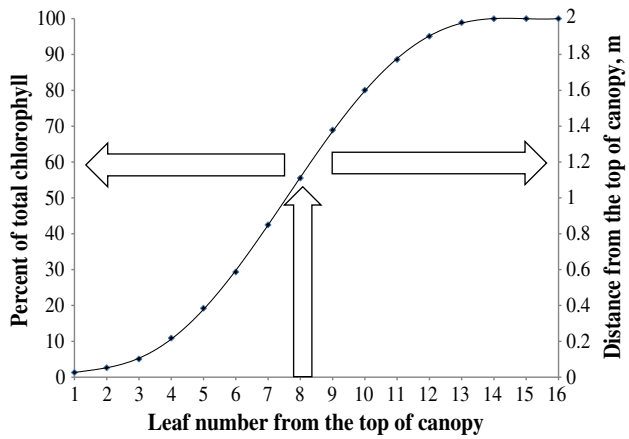

Fig. 8. Percent of total chlorophyll content in leaf layers from the top of canopy and distance from the top of canopy plotted versus leaf number from the top of canopy. Top leaf corresponds to No. 1. Layers of leaves from the top to ear leaf (No. 8), positioned around 1.2 m below the top of canopy and having maximal chlorophyll content, contain 55% to 65% of total canopy Chl content.

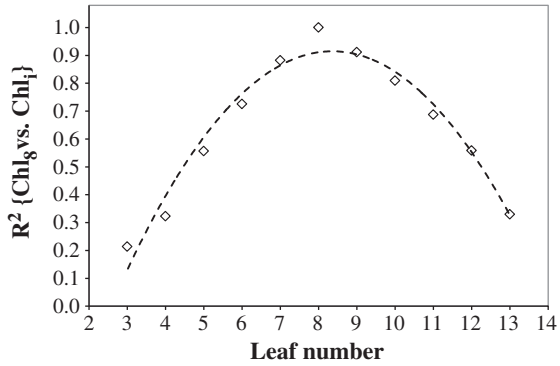

Fig. 9. Coefficient of determination of the linear relationship between Chl content in ear leaf, having maximal Chl content, and Chl content in other leaves versus leaf number — DOY 208, July 26. Top leaf corresponds to No. 1, ear leaf corresponds to No. 8.

the different hybrids and irrigation regimes, involved in this study, Chl content in the 8th leaf was closely related to Chl content in leaves positioned lower in the canopy (Fig. 9). The high sensitivity of $CI_{red\ edge}$ to Chl content in the upper 7–8 leaves and the close relation between 8th leaf Chl content and Chl content of leaves, positioned deeper in the maize canopy, provides an accurate estimation of total Chl content using the red-edge chlorophyll index.

5. Conclusion

Chlorophyll distribution in a maize canopy showed that (a) the ear leaf, located at the depth around 1.25 m from the top of the canopy, has maximal Chl content; (b) leaf layers from the top leaf to the ear leaf contain 55% to 65% of total maize Chl content; and (c) the top 11–12 leaf layers, located 1.5–1.7 m below the top of the canopy, contain 75% to 85% of total canopy Chl content and, thus, significantly contribute to total canopy Chl content.

The hierarchical regression procedure made it possible to assess the importance of the Chl content of each leaf in defining total Chl content in a maize canopy and documented very close relationships between red-edge chlorophyll index and total canopy Chl content when 8 to 10 top leaf layers were included in the model. In addition, three Aikaike Information Criteria identified the best model for $CI_{red\ edge}$ vs. canopy Chl content with top 7 to 9 leaf layers. Thus, statistical techniques showed that red-edge chlorophyll index, which employs the NIR and the red-edge (720–730 nm) spectral bands, senses the chlorophyll content of 7 to 9 upper leaves in a maize canopy. These 7 to 9 top leaf layers contain only 55% to 65% of total canopy Chl content. However, due to close relationships between Chl content in leaves, located in the middle of the canopy, red-edge chlorophyll index provides a very accurate estimate of maize canopy chlorophyll content.

Acknowledgments

We acknowledge the use of facilities and equipment provided by Center for Advanced Land Management Information Technologies (CALMIT), University of Nebraska-Lincoln. This research was supported in part by the U.S. Department of Energy EPSCoR program, Grant DE-FG-02-00ER45827; the Office of Science (BER), grant no. DE-FG03-00ER62996; NASA North American Carbon Program, grant NNX08AI75G; the University of Nebraska Agricultural Research Division, Lincoln, NE; and USDA-Agricultural Research Service.

References

Anderson, M. C. (1966). Stand structure and light penetration. 2. A theoretical analysis. *Journal of Applied Ecology*, *3*(1), 41–54.

Anderson, D. R., Burnham, K. P., & Thompson, W. L. (2000). Null hypothesis testing: Problems, prevalence, and an alternative. *Journal of Wildlife Management*, *64*(4), 912–923.

Barton, C. V. M. (2000). A theoretical analysis of the influence of heterogeneity in chlorophyll distribution on leaf reflectance. *Tree Physiology*, *21*, 789–795.

Ciganda, V., Gitelson, A. A., & Schepers, J. (2008). Vertical profile and temporal variation of chlorophyll in maize canopy: Quantitative "crop vigor" indicator by means of reflectance-based techniques. *Agronomy Journal*, *100*, 1409–1417, http://dx.doi.org/10.2134/agronj2007.0322.

Ciganda, V., Gitelson, A. A., & Schepers, J. (2009). Non-destructive determination of maize leaf and canopy chlorophyll content. *Journal of Plant Physiology*, *166*, 157–167.

Dwyer, L. M., Stewart, D. W., Hamilton, R. I., & Houwing, L. (1992). Ear position and vertical distribution of leaf area in corn. *Agronomy Journal*, *84*, 430–438.

Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia*, *78*, 9–19.

Gitelson, A. A (2011). Remote Sensing estimation of crop biophysical characteristics at various scales. Chapter 15 in Hyperspectral Remote Sensing of Vegetation (Thenkabail, P.S., Lyon, J.G., Huete, A., Eds). Taylor and Francis, pp. 329–358.

Gitelson, A. A., Gritz, U., & Merzlyak, M. N. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology*, *160*, 271–282.

Gitelson, A., & Merzlyak, M. N. (1996). Signature analysis of leaf reflectance spectra: Algorithm development for remote sensing of chlorophyll. *Journal of Plant Physiology*, *148*, 495–500.

Gitelson, A. A., Viña, A., Rundquist, D. C., Ciganda, V., & Arkebauer, T. J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical Research Letters*, *32*, L08403, http://dx.doi.org/10.1029/2005GL022688.

Gitelson, A. A., Viña, A., Verma, S. B., Rundquist, D. C., Arkebauer, T. J., Keydan, G., et al. (2006). Relationship between gross primary production and chlorophyll content in crops: Implications for the synoptic monitoring of vegetation productivity. *Journal of Geophysical Research*, *111*, D08S11, http://dx.doi.org/10.1029/2005JD006017.

Hornik (2006). The R FAQ. http://www.r-project.org ISBN 3-900051-08-9.

Keating, B. A., & Wafula, B. M. (1992). Modelling the fully expanded area of maize leaves. *Field Crops Research*, *29*, 163–176.

Kropff, M. J., & Goudrian, J. (1994). Competition for resource capture in agricultural crops. In J. L. Monteith, R. K. Scott, & M. H. Unsworth (Eds.), *Resource Capture by Crops*. Sutton Bonington Campus, Loughborough, Leicestershire: Nottingham University Press 1-897676-21-2.

le Maire, G., Francois, C., Soudani, K., Berveiller, D., Pontailler, J. -Y., Genet, H., et al. (2008). Calibration and validation of hyperspectral indices for the estimation of broadleaved forest leaf chlorophyll content, leaf mass per area, leaf area index and leaf canopy biomass. *Remote Sensing of Environment*, *112*, 3846–3864.

Peng, Y., Gitelson, A. A., Keydan, G. P., Rundquist, D. C., & Moses, W. J. (2011). Remote estimation of gross primary production in maize and support for a new paradigm based on total crop chlorophyll content. *Remote Sensing of Environment*, *115*, 978–989.

Tucker, C. J. (1977). Asymptotic nature of grass canopy spectral reflectance. *Applied Optics*, *16*, 1151–1156.

Rundquist, D., Perk, R., Leavitt, B., Keydan, G., & Gitelson, A. (2004). Collecting spectral data over cropland vegetation using machine-positioning versus hand-positioning of the sensor. *Computers and Electronics in Agriculture*, *2*, 173–178.

Ustin, S. L., Gitelson, A. A., Jacquemoud, S., Schaepman, M., Asner, G. P., Gamon, J. A., et al. (2009). Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. *Remote Sensing of Environment*, *113*, S67–S77, http://dx.doi.org/10.1016/j.rse.2008.10.019.

Valentinuz, O. R., & Tollenaar, M. (2004). Vertical profile of leaf senescence during the grain-filling period in older and newer maize hybrids. *Crop Science*, *44*, 827–834.

Westphal, M. I., Filed, S. A., Tyre, A. J., Paton, D., & Possingham, H. P. (2003). Effects of landscape pattern on bird species distribution in the Mt. Lofty Ranges, South Australia. *Landscape ecology*, *18*, 413–426.