

**LEAF PHOTOSYNTHETIC CAPACITY, CHLOROPHYLL CONTENT, AND  
SPECIFIC LEAF NITROGEN RELATIONSHIPS IN SUNFLOWER  
CROPS PRIOR TO ANTHESIS**

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**Summary:** Leaf area subroutines, widely used in crop simulation models, often estimate leaf area index (LAI) as a balance between leaf expansion and leaf senescence. In these models, leaf senescence estimations are often based on data derived from observations of leaf colour or chlorophyll content. The use of the chlorophyll decay as an indicator of changes in leaf assimilatory capacity is only valid if a good relation between chlorophyll and photosynthetic capacity holds. We investigated the relation between photosynthesis at high irradiance ( $P_{HI}$ ) and leaf chlorophyll in a sunflower crop grown at a commercial plant population density ( $4.76 \text{ plants m}^{-2}$ ) at Buenos Aires. During the period between achievement of maximum leaf size and leaf death of leaf #8, and at intervals between 2 and 5 days, we measured chlorophyll content using a chlorophyll meter (SPAD-502, Minolta, Plainfield, IL) and  $P_{HI}$  with a portable photosynthesis meter (Li-Cor 6200, LI-COR Inc, NE, USA).  $P_{HI}$  decreased 75% over a period of 15 days without any corresponding change in the leaf chlorophyll content being observed. Specific leaf nitrogen (Nf) exhibited a similar relationship with chlorophyll content in an experiment using sunflower canopies of contrasting plant population density (0.47 to  $4.76 \text{ plants m}^{-2}$ ). These findings strongly support the idea that estimations of assimilatory capacity based on chlorophyll decay calculations are not appropriate for sunflower. An alternative method to estimate  $P_{HI}$  is proposed based on Nf estimates derived from a Nf/light quality (red/far-red ratio) relationship.

## **Introduction**

During the recent years the development and use of crop growth and production simulation models has greatly increased. The major aim of these models is an accurate estimation of the crop yield which it is estimated from crop biomass production. In most simulation models, crop leaf area is one of the key variables for biomass production estimation (e.g., Jones and Kiniry 1986, Muchow and Sinclair 1994, Villalobos et al. 1996) and leaf area subroutines are based on the leaf area generation and senescence calculations.

Most biomass generation subroutines are based on the principle that crop radiation use efficiency is constant during all or sections of the crop season to simplify calculations, although it is known that canopy photosynthetic capacity decreases with leaf age. Leaf area subroutines usually consider the end of leaf life as defined by a decrease in chlorophyll content (e.g., Cock et al. 1979, Carberry et al. 1993). This is based on the assumption of linearity and proportional decrease in both photosynthesis and chlorophyll which would allow the use of chlorophyll as an indicator of leaf functionality. We tested the hypothesis that the decrease in leaf chlorophyll is a good predictor of leaf functionality for the basal leaves of sunflower crops.

## **Materials and Methods**

To test the leaf chlorophyll/functionality hypothesis we used data generated in experiments that are described in detail in previous publications (Rousseaux et al. 1996, Rousseaux et al. 1999).

### Exp. 1

#### *Growth conditions*

Sunflower seeds (cv. G100, Dekalb, Argentina) were sown on 6 October 1995, in the experimental field of the Facultad de Agronomía UBA (latitude  $34^{\circ} 35' S$ ,  $58^{\circ} 29' W$ ) at a density of 48 seeds  $m^{-2}$ . Final population density ( $4.76 \text{ plants } m^{-2}$ ; in rows 0.7 m apart and 0.3 m between plants in the row, the normal plant population density for commercial sunflower crops in Argentina) was established by thinning. Rows were oriented N-S. A randomized complete block design with 6 replications was used, and all plots (9 rows x 1.5 m) received  $25 \text{ kg N ha}^{-1}$  (calcium nitrate) at sowing and  $100 \text{ kg N ha}^{-1}$  (urea) in two doses. Soil water content was maintained near field capacity during the experiment using trickle irrigation. In this experiment we manipulated the light environment perceived by target leaves. We enriched ( $+ 8.33 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) the impinging red radiation of the abaxial surface of north-oriented 8th leaves (cotyledons =0) with red light emitting diode (LED) panels. Green and unlit LED panels were used as controls.

#### *Leaf chlorophyll and photosynthesis*

Leaf chlorophyll content was determined with a non-destructive chlorophyll meter (SPAD-502, Minolta, Plainfield, IL) every two days from end of leaf expansion (Rawson and Dunstone 1986) to senescence. For the calibration of the chlorophyll meter leaf 1-cm diameter disks were harvested from leaves of chlorophyllometer values and immediately placed in extracting solution. For the chlorophyll extraction we used NN dimethylformamide (Inskeep y Bloom 1985), obtaining the following regression.:

$$\text{Chlorophyll content (mg m}^{-2}\text{)} = -77.4 + 12.7 * \text{chlorophyll meter units} \quad (r^2 = 0.83, n=20) \quad \text{eq. 1}$$

Net carbon exchange rate ( $P_{HI}$ ) at high irradiances (average photosynthetically active

radiation impinging on target leaves during  $P_{HI}$  determinations was  $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$  ) of target leaves was measured, every 2-5 days using a portable photosynthesis system (Li-Cor 6200, LI-COR Inc, NE, USA). Measurements were performed at midday during sunny days, enclosing the apical third of the target leaf inside a 1L cuvette. Leaves were exposed to direct sunlight during at least 15 minutes before measurements were performed.

## Exp. 2

### *Growth conditions*

Sunflower seeds (cv. G100, Dekalb, Argentina) were sown on 7 Dec. 1995, in the experimental field of the Facultad de Agronomía UBA at a density of  $48 \text{ seeds m}^{-2}$ . Three plant populations were established by thinning 20 d after sowing (two fully expanded leaves): 0.47 (D1; plants equidistant, 1.5 m), 1.18 (D2; plants equidistant, 0.9 m) and  $4.76 \text{ plants m}^{-2}$  (D3; in rows 0.7 m apart and 0.3 m between plants in the row). Rows were oriented N-S. A randomized complete block design with five replications was used, and all plots received  $25 \text{ kg N ha}^{-1}$  (calcium nitrate) at sowing and  $75 \text{ kg N ha}^{-1}$  (urea) in two doses, 21 and 28 days after sowing. Soil water content was maintained near field capacity during the experiment.

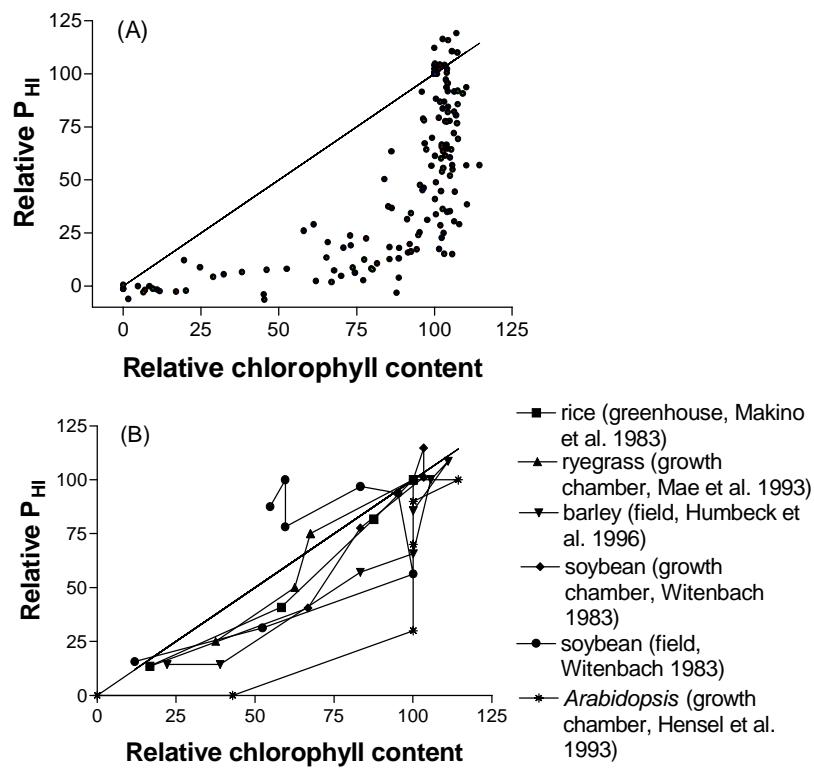
### *Leaf nitrogen content and chlorophyll content*

The basal quarters of N-oriented leaves (midrib within  $30^{\circ}$  of North), subtending the 12<sup>th</sup> node (0= cotyledons) leaves were harvested weekly from each plot for specific leaf nitrogen ( $N_f$ ,  $\text{g N m}^{-2}$ ) determinations. Leaves were oven dried at  $70^{\circ}\text{C}$  and milled using a Cyclotec sample mill (Tecator AB, Hoganas, Sweden) and their N content determined by micro-Kjeldahl technique using a Se catalyst and a Tecator Kjeltec Autosystem IV (Tecator AB, Hoganas, Sweden). Leaf chlorophyll content was determined with a non-destructive chlorophyll meter (SPAD-502, Minolta, Plainfield, IL) every two days from end of leaf expansion (Rawson and Dunstone 1986) to senescence.

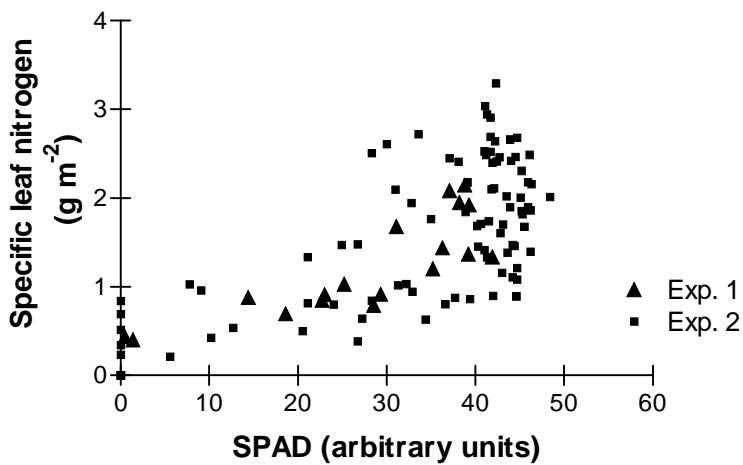
## **Results**

$P_{HI}$  decreased 75% over a period of 15 days without any corresponding change in the leaf chlorophyll content being observed (Exp. 1; Fig 1A). Similarly, other dicotyledonous species display a greater decrease in  $P_{HI}$  than in leaf chlorophyll during leaf senescence (Fig. 1B), although sunflower represents the most dramatic example with the possible exception of *Arabidopsis thaliana* (Fig. 1B).

Chlorophyll meter measurements are currently used to determine the nitrogen status of many crops (e.g., Fox et al. 1994, Turner and Jund 1994). To analyze whether chlorophyll meter readings can accurately predict  $N_f$  in senescent leaves of sunflower canopies we compared both variables in Exp. 1 and in an experiment using contrasting plant population densities (Exp. 2). Chlorophyll meter readings were not able to differentiate between  $N_f$  values greater than  $1.2 \text{ (g N m}^{-2}\text{)}$  because readings of around 40 units were related to leaves with  $N_f$  from 1.2 up to 3.5 (Fig. 2). This results suggest that chlorophyll determinations are not appropriate for  $N_f$  estimation in senescent sunflower leaves.



**Fig. 1:** P<sub>HI</sub> relative to the maximum value during the senescence period as a function of the corresponding relative chlorophyll data. Data corresponding to **(A)** sunflower (Exp. 1) and other **(B)** dicotyledonous and monocotyledonous species are represented. The straight line represents the 1:1 line. Each data point in Fig. 1 A corresponds to an individual leaf from the 8 th node measured during the senescence period (n=18).



**Fig. 2:** Measured specific leaf nitrogen ( $\text{g N m}^{-2}$ ) and chlorophyll meter readings for Exp. 1 and Exp. 2. Data correspond to individual leaves measured during the senescence period. (n= 18, Exp. 1; n= 90, Exp. 2).

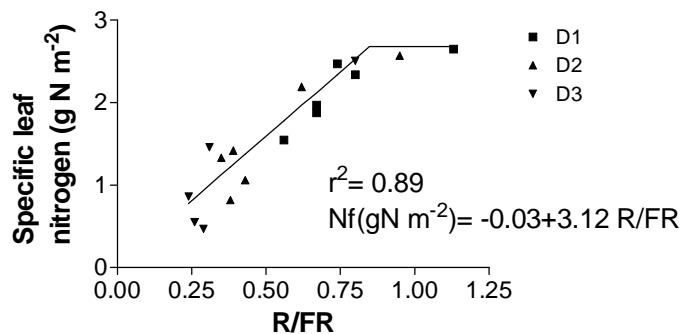
## Discussion

In crop simulation models, leaf area subroutines often estimate LAI as a balance between leaf

expansion and leaf senescence. In these models, leaf senescence estimations are often based on data derived from observations of leaf colour or chlorophyll content. We tested the validity of the use of chlorophyll content as an indicator of leaf functionality decay and found that this methodology can produce a significant overestimation of leaf photosynthetic capacity (Fig. 1). In the case of sunflower crops, chlorophyll content remained constant while  $P_{HI}$  decreased to less than 25% of its initial value at the end of leaf expansion. This finding points to the necessity of modifying the leaf senescence estimation in leaf area subroutines.

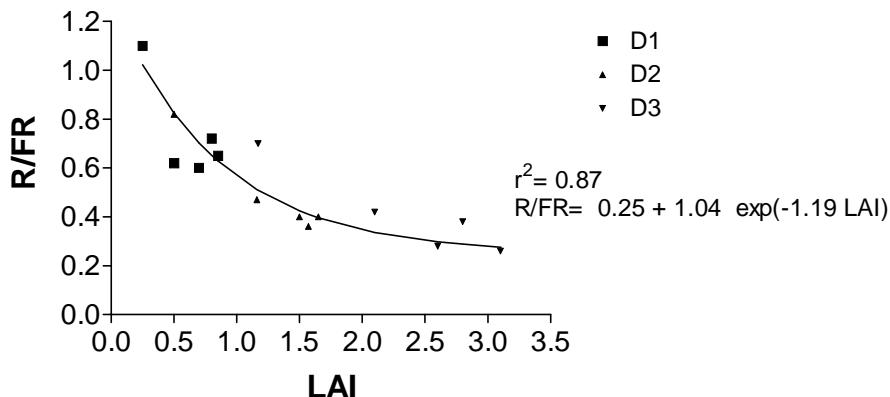
The uncoupling between photosynthetic capacity and chlorophyll content (Fig. 1A) may be explained by the uncoupling between the photosynthetic enzymes (which represent more than 70% of the leaf nitrogen content) and the chlorophyll content (Fig. 2), and suggest an alternative method which did not relate the  $P_{HI}$  to chlorophyll (directly or indirectly) should improve the canopy photosynthesis estimation in crop simulation models.

To improve the leaf senescence estimation in leaf area subroutines, it would be necessary to use a variable close related to leaf functionality. Also, it could be possible to replace the biomass estimation based on crop radiation use efficiency by a more mechanistic method. For example, Connor et al. (1993) proposed a method based on Sinclair et al. (1989) to estimated photosynthesis at light saturation ( $P_{max}$ ) as a function of  $N_f$ . For this to be feasible, a simple method for estimating  $N_f$  is required. The synthesis of many photosynthetic enzymes have been shown to respond to changes in light quality. In sunflower, c.i. 70% of leaf nitrogen is present in photosynthetic enzymes so it is expected that  $N_f$  is also control by changes in light quality. In a previous paper we showed that  $N_f$  increases linearly with R/FR ratio (eq. 2), for ratios between 0.2 and 0.85 (Rousseaux et al. 1999).



**Fig. 3:** Specific leaf nitrogen as a function of R/FR ratio perceived during the three days prior to leaf harvest for nitrogen determination for three contrasting plant population densities. Each point represent the average ( $n=5$ ) for each population density and harvest date (from Rousseaux et al. 1999).

Because of the optical properties of leaves, which absorb strongly in the R region of the spectrum and poorly in the FR region, R/FR ratio varies with leaf area index (LAI). We found that R/FR decreases exponentially as LAI increase (Fig. 4).  $N_f$  changes with PAR receipt have also been shown to exist, but in sunflower the  $N_f/R/FR$  relationship proved to be the best predictor of  $N_f$  within the canopy. If the relationship shown in Fig. 3 can be parameterized to deal with variations in total canopy N content, a combination of the descriptive functions shown in Figs. 3 and 4 may serve to describe  $N_f$  profiles within the canopy as a function of LAI.



**Fig. 4:** Relation between the R/FR ratio measured on the adaxial surface of the target leaf and the LAI above the target leaf for three contrasting plant population densities. Each symbol represent the average of 5 replication per density. The NON-LINEAR subroutine of the TBL program were used to fit the equation that explained most of the variability of the data.

To summarize, this research has shown that the use of leaf color or chlorophyll content can lead to errors in the estimation of functional LAI, and leaf area subroutines based on observations of this nature can lead to overestimates of functional LAI in crop simulation models. On the basis of the robustness of the Nf/R/FR relationship, it is speculated that relationships of this nature may serve as a better basis for describing Nf profiles in the canopy, and hence, canopy functionality.

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