PHOTOSYNTHETICA 46 (2): 294-297, 2008

BRIEF COMMUNICATION

Evaluation of a leaf area prediction model proposed for sunflower

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Abstract

Six leaf samplings were conducted in two sunflower (*Helianthus annuus* L.) hybrids during the 2006 growing season in order to evaluate a simple model proposed for leaf area (LA) estimation. A total of 144 leaves were processed using an image analysis system and LA, maximum leaf width (W) [cm], and midvein length (L) [cm] were measured. Also, LA was estimated using the model proposed by Rouphael *et al.* (2007). Measured LA was exponentially related with L and W, and the W–LA relationships showed higher r^2. Estimated LA was strongly and exponentially related with L. Strong, linear relationships with high r^2 between estimated and measured LA confirmed the high predictability of the proposed model.

Additional key words: *Helianthus annuus*; leaf length; leaf width; non-destructive methods.

Sunflower (*Helianthus annuus* L.) is a crop producing oil for both human consumption and industrial uses. In Greece, sunflower crop is restricted but it has recently gained interest for bio-diesel production and its cultivation is expected to expand. Thus, crop and especially its physiology has been the subject of very limited studies in Greece. Leaf area (LA) at individual or crop level is a parameter related with physiological processes such as growth and its partitioning, photon capture, and photosynthesis. Leaf Area Index (LAI) defined as the total leaf area [m^2] per m^{-2}(ground area) is a very important parameter for photon interception and yield formation and participates in models used for yield predictions (Yin *et al.* 2000, Launay and Guérif 2003, Rosenthal and Vanderlip 2004). Moreover, leaf expansion rate under stressful conditions could be used as a criterion for selecting tolerant genotypes (Ober and Luterbacher 2002). However, LA estimation, especially in the field, is very laborious and time-consuming or demands contemporary, high-cost equipment for non-destructive determination. Thus quick, easy, accurate, and successive estimation of LA under field conditions is of significant importance for agronomists, breeders, and physiologists. Lu *et al.* (2004) proposed that simple and especially linear relationships between LA and leaf dimensions (length, L and width, W) could be useful for non-destructive estimation of LA. Till now, non-destructive models for LA determination have been established for many species such as maize (Stewart and Dwyer 1999), bean (Bhatt and Chanda 2003), taro (Lu *et al.* 2004), white clover (Gamper 2005), sugar beet (Tsialtas and Maslaris 2005, 2008), sunflower (Květ and Marshall 1971, Rouphael *et al.* 2007), radish (Salerno *et al.* 2005), zucchini (Rouphael *et al.* 2006), strawberry (Demirsoy *et al.* 2005), grapevines (Manivel and Weaver 1974, Montero *et al.* 2000, Williams and Martinson 2003), kiwi (Mendoza-de Gyves *et al.* 2007), chestnut (Serdar and Demirsoy 2006), and hazelnut (Cristofori *et al*. 2007).

Since a simple model for sunflower LA determination has already been established (Rouphael *et al.* 2007), the aim of this work was to compare estimated and measured LA of two sunflower hybrids during successive samplings and to relate them with measured leaf dimensions (length, L and width, W).

Received 4 May 2007, *accepted* 19 October 2007.
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Abbreviations: ANOVA – analysis of variance; CV – coefficient of variation; L – leaf midvein length; LA – leaf area; LAI – leaf area index; LSD – least significant difference; W – maximum leaf width.
Acknowledgments: We thank Mr A. "Zucchero" Zaharos for his help during the experimentation and Mr K. Ikonomidis, Syngenta Hellas SA, Anthoussa Attiki, Hellas for providing the seeds.

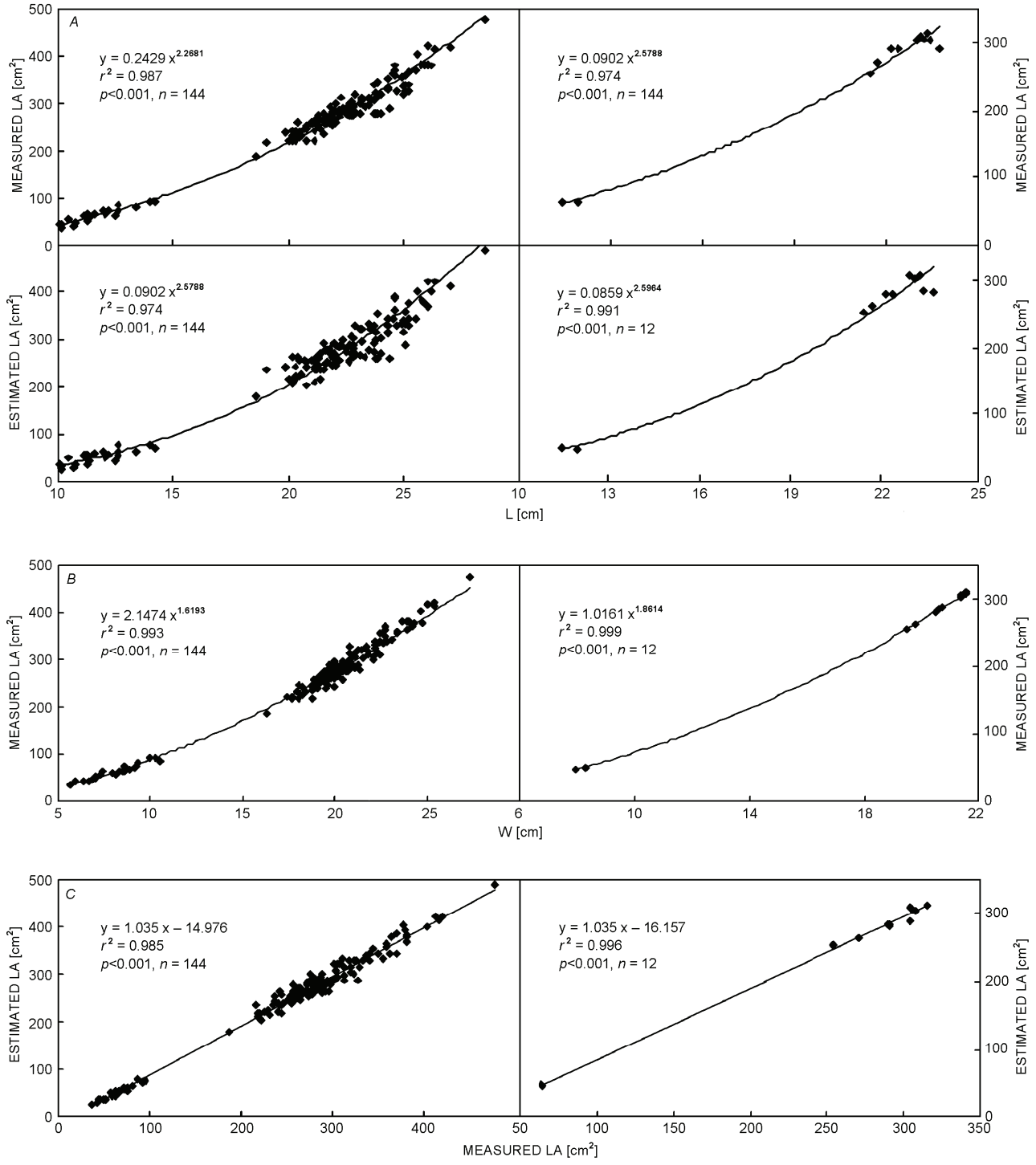

Fig. 1. Best-fitted curves of the relationships between (*A*) leaf length – L and leaf area – LA (measured or estimated), (*B*) leaf width – W and measured LA, and (*C*) measured and estimated LA.

Two sunflower hybrids Sunbro and Sunluca-RM (*Syngenta Hellas SA*, Anthoussa Attiki, Hellas) were mechanically sown on 15 June 2006 in six rows (7-m long) per plot, at 50 cm apart and at 20 cm spacing in the row. The study site was located in Larissa, Thessaly, Greece (39º40'2N, 22º27'1E, 70 m a.s.l.), on a sandy-loam soil, with pH 7.9, $CaCO_3$ 59 g kg^{-1}, organic matter 0.75 %, total N 0.7 g kg^{-1}, NO_3-N 18 mg kg^{-1}, and P-Olsen 9.7 mg kg^{-1}. Fertilization was applied as basal [100 kg(N) ha^{-1} and 41.5 kg(P) ha^{-1}] and top-dressing

[50 kg(N) ha^{-1}]. Five days after seeding, irrigation was applied in order to facilitate seedling emergence. Supplemental irrigation was applied when necessary.

The experiment was arranged as a Randomised Complete Block design with four replications. Leaf samplings took place at six times during the growing season (July 17th and 28th, August 8th, 17th, and 24th, and September 7th). On each occasion, three randomly selected, fully-expanded, upper leaves were sampled per plot. The leaves were sealed in plastic bags, put on an iced chest and transferred immediately to the Physiology Laboratory, Larissa factory, Hellenic Sugar Industry SA for determinations. Maximum W, midvein L, and LA were measured using the *WinDias* image analysis system (*Delta-T Devices*, Cambridge, UK). L was measured from the lamina tip to the point of lamina-petiole intersection and W was the maximum distance between the lamina lobes perpendicularly to the mid-rib. A total of 144 leaves were used for the determination of the leaf parameters. Also, LA was estimated using the linear equation proposed by Rouphael *et al*. (2007):

$$LA = 6.72 + 0.65\ W^2$$

The data (estimated and measured LA, L, W) were subjected to ANOVA as a Randomized Complete Block design with sampling dates and cultivars as main factors. Mean values were compared with LSD test at $p<0.05$. Statistical analysis was carried out with *MSTAT-C* (version *1.41*, Crop and Soil Sciences Department, Michigan State University, USA). Correlation analyses (linear, logarithmic, polynomial, hyperbolic, and exponential) between the parameters were assessed using *SPSS 14.0*, and graphs were constructed using *Excel 98* software (*MSOffice*, *Microsoft*).

ANOVA revealed significant effects of sampling date and sampling date×cultivar interaction on all determined parameters (estimated and measured LA, L, and W). However, the effect of sampling date on all parameters was not significant from the second sampling date onward. No cultivar effect was found (Table 1). Means (n = 12) and total data (n = 144) were used for establishing relationships between the parameters. The criterion of selecting the best-fitted curves was the higher r^2 (Rouphael *et al.* 2007). For both measured and estimated LA, the best-fitted curves between LA and L or W were those of exponential functions (Fig. 1). The W–LA relationships showed higher r^2 in accordance with previous reports for sunflower (Rouphael *et al*. 2007) or other species (Tsialtas and Maslaris 2005). Strong, exponential relationships were found between L and estimated LA (Fig. 1) but r^2 was comparatively low. A weakness of our data set is the gap between small and large leaves, which was the result of the fast expansion rate observed between the first and the second samplings. However, even excluding the smaller leaves, the relationships maintained their high significance and r^2 (data not shown).

Table 1. ANOVA of the determined parameters. not significant = ns ($p>0.05$); $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$.

Source of variation	Estimated LA	Measured LA	L	W
Blocks	***	***	**	***
Sampling dates (S)	***	***	***	***
Cultivars (C)	ns	ns	ns	ns
S×C	**	*	*	*
CV [%]	10.43	10.07	4.24	5.14

Measured and estimated LA were linearly related for both means and individual measurements (Fig. 1) meaning that the proposed model could predict sunflower LA rather accurately. However, the highly significant, with a very high r^2 relationships reported in this work could also provide reliable LA estimations. The main advantage of the model proposed by Rouphael *et al.* (2007) is its simplicity since it is a linear relationship (Lu *et al.* 2004, Tsialtas and Maslaris 2008).

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