Nitrogen fertilization effects on leaf morphology and evaluation of leaf area and leaf area index prediction models in sugar beet

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Abstract

In a two-year experiment (2002–2003), five N application rates [0, 60, 120, 180, and 240 kg(N) ha^{-1}, marked N_0, N_{60}, N_{120}, N_{180}, and N_{240}, respectively] were applied to sugar beet cv. Rizor arranged in a Randomized Complete Block design with six replications. Leaf shape parameters [leaf area (LA), maximum length (L), maximum width (W), average radial (AR), elongation (EL), and shape factor (SF)] were determined using an image analysis system, and leaf area index (LAI) was non-destructively measured every two weeks, from early August till mid-September (four times). Years, samplings, and their interaction had significant effects on the determined parameters. Fertilization at the highest dose (N_{240}) increased L and sampling×fertilization interaction had significant effects on LA, L, W, and SF. For this interaction, W was the best-correlated parameter with LA and LAI meaning that W is a good predictor of these parameters. Two proposed models for LA estimation were tested. The model based on both leaf dimensions [LA = 0.5083 (L×W) + 31.928] predicted LA better than that using only W (LA = 21.686 W – 112.88). Instrumentally measured LAI was highly correlated with predicted LAI values derived from a quadratic function [LAI = –0.00001 $(LA)^2$ + 0.0327 LA – 2.0413]. Thus, both LA and LAI can be reliably predicted non-destructively by using easily applied functions based on leaf dimensions (L, W) and LA estimations, respectively.

Additional key words: *Beta vulgaris*; leaf area; leaf length; leaf width; non-destructive methods.

Introduction

Sugar beet shows different leaf morphology depending on cultivar ploidy (Bosemark 1993) and environmental effects (Tsialtas and Maslaris 2007). Leaf shape determination can be useful for both taxonomic (Iwata *et al.* 2002, Camargo Neto *et al.* 2006) and physiological (Goudriaan and van Laar 1994, Bhatt and Chanda 2003) studies.

Leaf area (LA) is the most commonly determined leaf shape parameter. As regards sugar beet physiology, LA is related with photon harvesting *via* its effect on leaf area index (LAI) (Scott and Jaggard 1993) and could serve as a screening tool for genotypic drought tolerance (Ober and Luterbacher 2002). LA determination under field conditions is subjected to time and labour restrictions or needs investment in expensive, sophisticated equipment. Thus, non-destructive models based on leaf dimensions (L, W) were proposed in order to overcome easily the above-mentioned restrictions. Such models have been developed for many field crops such as beans (Bhatt and Chanda 2003), castors (Wendt 1967), cotton (Johnson 1967, Wendt 1967), faba bean (Peksen 2007), groundnut (Kathirvelan and Kalaiselvan 2007), maize (Stewart and Dwyer 1999), pearl millet (Payne *et al.* 1991), sorghum (Wendt 1967), sugar beet (Tsialtas and Maslaris 2005, 2008), safflower (Çamaş *et al.* 2005), sunflower (Bange *et al.* 2000, Rouphael *et al.* 2007), taro (Lu *et al.* 2004),

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Abbreviations: ANOVA – analysis of variance; AV – average radial; CEC – cation exchange capacity; CV – coefficient of variation; EC – electrical conductivity; EL – elongation; L – maximum length; LA – leaf area; LAI – leaf area index; LSD – least significant difference; SF – shape factor; W – maximum width.
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and white clover (Gamper 2005). For practical reasons, linear, one-dimensional models are more preferable (Lu *et al.* 2004, Tsialtas and Maslaris 2005).

LAI, the ratio of LA [m^2] per m^2 ground, is a constitutive parameter of many yield-predicting models since it is related with radiation interception (Yin *et al.* 2000, Launay and Guérif 2003). However, its use in field physiology studies is restricted due to reasons analogous to those referred to LA determination in the field. Thus, non-destructive models for LAI estimation based on LA measurements have already been developed for beans (de Jesus *et al*. 2001), tomato (Blanco and Folegatti 2003), and sugar beet (Tsialtas and Maslaris 2007).

Evaluation of models proposed for LA and LAI estimation under different environmental or growing conditions is necessary since leaf morphology is affected by growth, nutrition, and temperature (Njoku 1957, Bhatt and Chanda 2003, Tsialtas and Maslaris 2007). Till now, models based on W and LA were proposed for LA and LAI estimation, respectively, for sugar beet cv. Rizor (Tsialtas and Maslaris 2005, 2007). Also, a model based on both leaf dimensions (L×W) was the best-fitted for LA prediction in different sugar beet cultivars (Tsialtas and Maslaris 2008).

The aim of this work was to study the effect of five N application rates [0, 60, 120, 180, and 240 kg(N) ha^{-1}, marked N_0, N_{60}, N_{120}, N_{180}, and N_{240}, respectively] on leaf shape parameters (leaf area – LA, maximum length – L, maximum width – W, average radial – AR, elongation – EL, shape factor – SF) and LAI of sugar beet cv. Rizor. Also, the relationships between leaf dimension parameters and LA or LAI were established and models proposed for LA or LAI prediction were tested.

Materials and methods

Experimental site and set up: For two years (2002 and 2003), sugar beet cv. Rizor (*SESVANDERHAVE* NV/SA, Tienen, Belgium) was arranged in a Randomized Complete Block design experiment with five N application rates and six replications. The main aim of the experiment was to study the effect of N fertilization on sugar beet yield and quality. The experiment was located on a typical clayey inorganic soil of eastern Thessaly Plain (39º33´N, 22º27´E, 98 m asl). Table 1 presents some soil characteristics in 0–0.30 m depth before experiment establishment.

Seeding was conducted mechanically (19 March 2002 and 18 March 2003) in eight rows (8-m long) per plot, at 0.5 m apart and at 0.15 m spacing in the row. The 2 : 3 of N were applied as basal (ammonium sulphate) and the rest as top-dressing (ammonium nitrate). Before sowing, 90 kg(P) ha^{-1} (hyper-phosphate) and 265 kg(K) ha^{-1} (potassium sulphate) were incorporated into the soil. During the growing season, full protection was taken against cercospora leaf spot, powdery mildew, weeds, and insects by chemical sprayings. Supplemental irrigation was provided according to the needs and availability of irrigation water. The total water input [rainfall + irrigation] was 610 and 498 mm in 2002 and 2003, respectively.

Parameter determination: In both years, four leaf samplings were conducted every two weeks beginning in early August. Three upper, healthy and fully expanded sunlit leaves were collected from each plot, sealed in a plastic bag and put in a portable refrigerator. The samples were transferred to the Physiology Laboratory of Larissa factory, Hellenic Sugar Industry SA, for determinations. Leaf shape parameters (LA, L, W, AR, EL, and SF) were measured using the *WinDias* image analysis system (*Delta-T Devices*, Cambridge, UK). Each year, 360 individual leaves were measured. The average of the three measurements comprised the value of one replication. LAI was determined non-destructively using the *SunScan* canopy analysis system (*Delta-T Devices*, Cambridge, UK). Two measurements were taken between the 4^{th} and 5^{th} rows in each plot and the average was calculated. In case of deviated measurements, a third LAI determination was conducted.

Calculations and statistics: LA was estimated using Model 1 which was proposed for cv. Rizor [LA = 21.686 W – 112.88] and Model 2 proposed for various sugar beet cultivars [LA = 0.5083 (L×W) + 31.928] (Tsialtas and Maslaris 2005, 2008). Based on LA measurements, LAI was estimated as follows (Tsialtas and Maslaris 2007): $LAI = -0.00001\,(LA)^2 + 0.0327\,LA - 2.0413$.

The data were subjected to *ANOVA* as a Randomized Complete Block design with years as the main factor and samplings and N application rates as split factors. Means were compared with the *LSD* test at $p<0.05$. Statistical analysis was carried out with the *MSTAT-C* package (version *1.41*, Crop and Soil Sciences Department, Michigan State University, USA). Figures were displayed using *Excel 98* software (*MSOffice, Microsoft*) and the significance level of the correlations was determined by *SPSS 14*.

Results and discussion

Leaf shape parameters and LAI were significantly affected by years, samplings, and their interaction. No year effect was evident for EL (Table 2). The traits determined were higher in 2002 compared to 2003. A gradual decline with the progress of time was found for LA, L, W, AR, and EL with the trend being more pronounced in 2002.

On contrary, only small, insignificant changes of the leaf parameters were found in 2003, when the leaves were smaller (Table 3). SF increased significantly with time and the highest values were found between mid-August (2002) and early September (2003). In both years, LAI was highest in early August and then declined gradually in 2002 and abruptly in 2003 (Table 3).

Table 1. Some soil characteristics at 0–0.30 m depth before the establishment of the experiment. CEC – cation exchange capacity, EC – electrical conductivity.

Year	pH [1:1]	Total $CaCO_3$ [g kg^{-1}]	Org. matter [g kg^{-1}]	CEC [cmol kg^{-1}]	EC [dS m^{-1}]	Total N [g kg^{-1}]	NO_3-N [m kg^{-1}]	P-Olsen [m kg^{-1}]
2002	7.8	10.2	14.9	43.9	0.52	1.2	5.7	32.9
2003	8.1	56.2	13.8	37.6	0.89	1.5	9.7	5.7

Table 2. Analysis of variance (*ANOVA*) of leaf shape parameters and LAI. ns: not significant; *, **, ***significance at p<0.05, <0.01, 0.001, respectively; LA: leaf area; L: maximum length; W: maximum width; AR: average radial; EL: elongation; SF: shape factor; df: degrees of freedom; CV: coefficient of variation.

Source of variation	df	LA	L	W	AR	EL	SF	LAI
Blocks	5	ns	ns	ns	ns	ns	ns	Ns
Years (Y)	1	***	***	***	***	ns	***	**
Samplings (S)	3	***	***	***	***	***	***	***
Y×S	3	***	***	***	***	***	***	***
Fertilization (F)	4	ns	*	ns	ns	ns	ns	ns
Y×F	4	ns	ns	ns	ns	ns	ns	ns
S×F	12	*	*	*	ns	ns	*	ns
Y×S×F	12	ns	ns	ns	ns	ns	ns	ns
CV [%]		9.10	5.15	6.32	4.67	7.50	4.99	32.06

Table 3. Comparison of means of the leaf shape parameters and LAI determined for the year×sampling interaction. LA: leaf area; L: maximum length; W: maximum width; AR: average radial; EL: elongation; SF: shape factor; e-, m-Aug: early, mid-August; e-, m-Sept: early, mid-September. For each trait, means labelled with the same letter did not differ significantly.

Date	LA [cm^2] 2002	LA [cm^2] 2003	L [cm] 2002	L [cm] 2003	W [cm] 2002	W [cm] 2003	AR [cm] 2002	AR [cm] 2003	EL 2002	EL 2003	SF 2002	SF 2003	LAI 2002	LAI 2003
e-Aug.	211.1 a	129.5 e	22.16 a	18.52 d	15.44 a	11.30 e	8.40 a	6.68 e	0.701 a	0.615 cd	2.46 c	1.95 f	4.65 a	4.28 a
m-Aug.	183.8 b	125.8 e	21.01 b	18.20 de	14.32 b	11.28 e	7.88 b	6.56 ef	0.685 a	0.622 bc	2.74 a	1.98 f	3.69 b	3.06 cd
e-Sept.	156.2 c	124.0 e	20.84 b	18.06 de	12.28 c	11.16 e	7.57 c	6.54 ef	0.593 de	0.622 bc	2.58 b	2.13 d	3.35 bc	1.33 e
m-Sept.	142.0 d	123.7 e	19.93 c	17.79 e	11.74 d	11.37 de	7.07 d	6.48 f	0.591 e	0.643 b	1.84 g	2.07 e	2.68 d	1.28 e

Table 4. Correlation coefficients and significance level of the linear relationships between leaf shape parameters, LA and LAI for the sampling×fertilization interaction. *, **, ***: significant coefficients at p<0.05, <0.01, or <0.001, respectively; n = 20; LA – leaf area, L – maximum length, W – maximum width, AR – average radial, EL – elongation, SF – shape factor.

	W [cm]	AR [cm]	EL	SF	LA [cm^2]	LAI
L [cm]	0.947 x – 6.1659 0.77***	0.4274 x – 121.45 0.93***	ns	ns	20.711 x – 255.69 0.88***	1.2044 x – 20.523 0.78***
W [cm]		0.3404 x + 2.9387 0.92***	0.0288 x + 0.2782 0.86***	ns	18.565 x – 79.996 0.97***	1.1916 x – 11.691 0.95***
AR [cm]			0.0551 x + 0.2401 0.61**	0.2576 x + 0.3751 0.45*	50.26 x – 209.71 0.98***	1.4924 x – 8.0444 0.91***
EL				ns	417.39 x – 115.2 0.73***	12.393 x – 5.2374 0.78***
SF					ns	ns
LA [cm^2]						0.0622 x – 6.2557 0.95***

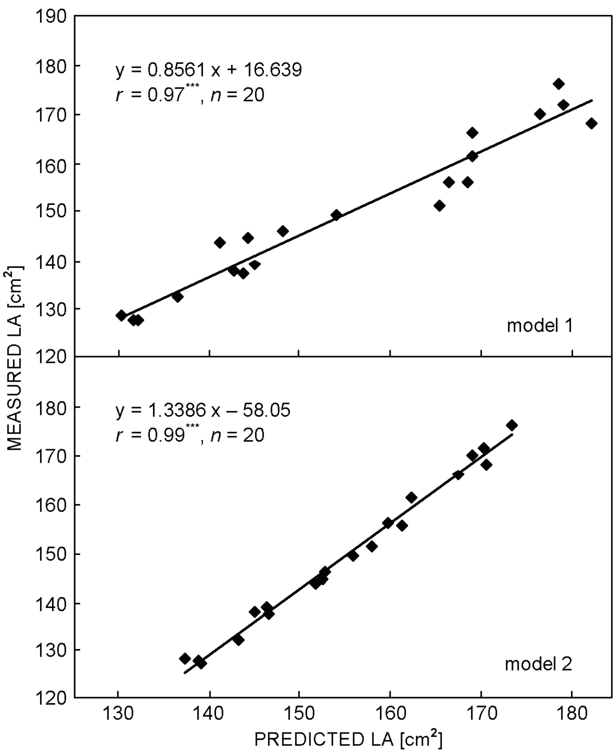

Fig. 1. Coefficients and significance of the correlation between predicted and measured LA for sampling×fertilization interaction. Model 1: LA = 21.686 W – 112.88; Model 2: LA = 0.5083 (L×W) + 31.928; ns: not significant; ***: significant coefficients at $p<0.001$.

Our findings are indicative of heteroblasty, *i.e.* changes of leaf shape that occur during development (Byrne *et al.* 2001) in sugar beets confirming a previous report (Tsialtas and Maslaris 2007). Leaf morphology is affected by both genetic and environmental factors (Kessler and Sinha 2004) such as nutrient and water availability and temperature (Njoku 1957, Tsialtas and Maslaris 2007). As regards sugar beet, leaf morphology showed a plastic reaction to water availability (Tsialtas and Maslaris 2007) and this mechanism can represent an adaptive advantage in different environments (Sultan 2000).

Fertilization had a significant effect only on L for which the highest values were found for the highest N dose (N_{240}). However, the sampling×fertilization interaction was significant for LA, L, W, and SF (Table 2). For this reason, correlations between leaf dimension parameters and LA or LAI were established (Table 4). Leaf L was significantly correlated with W, AR, LA, and LAI. The respective correlations between W and LA or LAI showed higher coefficients in accordance with previous reports of W being a best predictor of LA and LAI (Williams and Martinson 2003, Tsialtas and Maslaris 2005, 2007, Rouphael *et al.* 2007).

Our results confirmed the feasibility of LAI estimation based on LA measurements in sugar beet (Tsialtas and Maslaris 2007). Analogous findings have already been reported for beans (de Jesus *et al.* 2001) and tomato (Blanco and Folegatti 2003). In sugar beet, instrumental, non-destructive LAI estimation was highly correlated with destructive LAI measurements (Röver and Koch 1995) but the high cost restricts equipment acquisition and thus LAI estimation in field crop researches. AR showed a high correlation with leaf dimensions (L, W), LA, and LAI, but it could not be useful due to its difficult determination in the field (Tsialtas and Maslaris 2007). The positive correlation between EL and LA or LAI means that leaves became more rounded when they were more expanded and sugar beet had high LAI values (Table 4).

Two linear models were proposed for LA prediction in sugar beet. Model 1 is based on W estimation and proposed for LA prediction in cv. Rizor and Model 2 uses both leaf dimensions (L×W) for LA prediction for different sugar beet cultivars (Tsialtas and Maslaris 2005, 2008). Model 2 predicted LA more accurately since its coefficient of correlation between predicted and measured LA was higher ($r = 0.99$) than the respective one found for Model 1 ($r = 0.97$) (Fig. 1). Similarly, the quadratic function proposed for LAI prediction in sugar beet cv. Rizor gave values highly correlated ($r = 0.95$) with those measured instrumentally (Fig. 2).

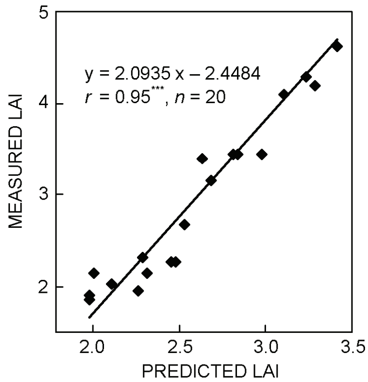

Fig. 2. Coefficients and significance of the correlation between predicted and measured LAI for sampling×fertilization interaction. Predicted LAI was estimated as: LAI = –0.00001 $(LA)^2$ + 0.0327 LA – 2.0413. ***: significant coefficient at $p<0.001$.

In conclusion, years, samplings, and their interaction affected significantly leaf shape parameters. Nitrogen fertilization (N_{240}) affected positively only L but the sampling×fertilization interaction was significant for LA, L, W, and SF. For the former interaction, W was highly and linearly correlated with LA and LAI meaning that it was a good predictor of these parameters. LA predicted by a proposed model [LA = 0.5083 (L×W) + 31.928] was highly correlated with the measured values. Also, LAI was accurately predicted by a proposed quadratic function [LAI = –0.00001 $(LA)^2$ + 0.0327 LA – 2.0413].

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Appendix

Average Radial (AR): The average of all the distances measured from the centroid to each perimeter point.
Centroid (CY or CX): The most central point or the centre of gravity of the object (measured from the top left-hand corner of the screen).
Elongation (EL): The ratio of width and length.
Shape Factor (SF): The ratio of the actual perimeter to that of a circle with the same area

$$SF = P/Pc,$$

where P is the perimeter of the object and Pc is the perimeter of a circle with the same area as the object.