# Leaf shape and its relationship with Leaf Area Index in a sugar beet (*Beta vulgaris* L.) cultivar

J.T. TSIALTAS* and N. MASLARIS**

*Hellenic Sugar Industry SA*
**Larissa factory, Department of Experimentation, 411 10 Larissa, Hellas*
***Agronomic Research Service, 547 00 Sindos, Hellas*

## Abstract

Heteroblasty of sugar beet cultivar Rizor was studied under field conditions for three growing seasons (2003, 2005, 2006) in a Randomized Complete Block (RCB) design experiment. Eleven leaf samplings, from early June till the end of October, were conducted each year and leaf shape parameters [leaf area (LA), centroid X or Y (CX or CY), length (L), width (W), average radial (AR), elongation (EL), shape factor (SF)] were determined by an image analysis system. During samplings, Leaf Area Index (LAI) was measured non-destructively. Significant year and sampling effects were found for all traits determined. With the progress of the growing season, leaves became smaller (LA, L, W, and AR were decreased) and rounded. The largest leaves were sampled in 2006 when LAI was highest. LA was strongly correlated with L and W with simple functions ($y = 0.1933\ x^{2.2238}$, $r^2 = 0.96$, $p<0.001$, and $y = 28.693\ x - 192.33$, $r^2 = 0.97$, $p<0.001$, respectively), which could be used for non-destructive LA determination. Also, LAI was significantly related with LA and leaf dimensions (L, W) suggesting that an easy, non-destructive determination of LAI under field conditions is feasible for sugar beet cv. Rizor.

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

## Introduction

Sugar beet cultivars are diploid and triploid hybrids differing in their leaf shape and morphology (Bosemark 1993). Development of plant morphology is gene regulated and environmentally affected (Iwata *et al.* 2002a,b, Kessler and Sinha 2004). Quantitative evaluation of organ shape is often needed for many field researches in agronomy, zoology, genetics, ecology, and taxonomy (Iwata and Ukai 2002). Due to its inherent character, leaf shape could be used for plant species identification (Camargo Neto *et al.* 2006, Du *et al.* 2007) or cultivar classification (Iwata *et al.* 2002a). LA estimation is a useful parameter for plant growth analysis and evapotranspiration studies (Bhatt and Chanda 2003). In sugar beet, leaf area is a trait related with photosynthesis and LAI (Scott and Jaggard 1993). Also, determination of LA in successive periods during growing season (relative leaf expansion rate) could be used as a screening tool for drought tolerance of sugar beet cultivars (Ober and Luterbacher 2002, Ober *et al.* 2005).

Although quantitative evaluation of biological organ shape is easy using computer-aided determination (Iwata and Ukai 2002), expensive hardware and sophisticated software is necessary and its use is restricted (Korva and Forbes 1997). Thus, easily applied, non-destructive methods based on simple equations have been developed for organ shape (mainly LA) determination in bean (Bhatt and Chanda 2003), grapevine (Williams and Martinson 2003), taro (Lu *et al.* 2004), white clover (Gamper 2005), and sugar beet (Tsialtas and Maslaris 2005). Non-destructive, mathematical methods of LA estimation could substitute for laborious and time-consuming methods especially under field conditions (Lu *et al.* 2004).

Seasonal development and final value of LAI, the ratio of LA [$m^2$] per $m^2$ ground, is of crucial importance for crop productivity due to its determinant role in radiation interception. Thus, LAI is an important

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*Corresponding author; *present address*: NAGREF, Cotton and Industrial Plants Institute, 574 00 Sindos, Hellas, fax: +30 2310 796513, e-mail: tsialtas01@hotmail.com
*Abbreviations*: AR – average radial; C – centroid; EL – elongation; L – length; LA – leaf area; LAI – Leaf Area Index; LSD – least significant difference; RCB – Randomized Complete Block; SF – shape factor; W – width.
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parameter in crop production models (Yin *et al.* 2000, Launay and Guérif 2003, Rosenthal and Vanderlip 2004).

In sugar beet crop, a LAI of *ca*. 3.5–4.0 is necessary for maximizing photon interception and yield (Scott and Jaggard 1993). Similar to LA, non-destructive, instrumental methods for LAI determination have been evolved and are widespread for field research (Jonckheere *et al.* 2004, Weiss *et al.* 2004). In sugar beet, instrumental, non-destructive LAI measurement was highly related with destructive LAI measurements or with optical remote sensing data (Röver and Koch 1995, Hoffmann and Blomberg 2004). Thus, easily applied, non-destructive methods of LAI determination is of increasing interest in sugar beet field research. However, we are unaware of any work on relating leaf shape parameters to non-destructive LAI determination in sugar beet. An analogous work is reported for bean in which non-destructively measured LAI was related with LA of the central leaflet of the trifoliate leaf (de Jesus *et al*. 2001).

The aim of this work was to explore the relationships between seasonal changes in leaf shape parameters with instrumentally measured LAI in sugar beets grown under field, Mediterranean conditions.

## Materials and methods

Cultivar Rizor (*SES EUROPE NV/SA*, Tienen, Belgium) was established in a Randomized Complete Block design with 6 replications in a long-term experiment aiming to study the year effects on root and sugar yield. The experiments were conducted, in adjacent sites, 22 km SW from Larissa factory (39º40’2N, 22º27’1E) of Hellenic Sugar Industry SA for three years (2003, 2005, 2006). Seeds were mechanically drilled in 6 rows (8-m long) per plot, at 50 cm apart, and at 15 cm spacing in the row. Seeding was done between 18 and 24 March. The soil was calcareous (pH >8.0), heavy (clay content >45 %), with total N >1 g $kg^{-1}$ and low organic matter content (<2 %). Adequate fertilization [per ha] 150 kg N, 90 kg P, and 265 kg K was applied as basal and top-dressing. Supplementary irrigation was provided according to the needs of the crop. During growing season, full protection was taken against cercospora, powdery mildew, weeds, and insects by sprayings. Mean temperature and rainfall during the growing season are given in Table 1.

Each year, starting from the beginning of June and every two weeks till November, 11 samplings were done. LAI was non-destructively determined between the 3$^{rd}$ and 4$^{th}$ rows in each plot and sampling date using *SunScan* system (*Delta-T Devices*, Cambridge, UK). Due to high repeatability, two measurements were taken in each plot and the mean was estimated. In case of deviated measurements, a third LAI determination was conducted. Concurrently, representative, upper, full-expanded, and healthy leaves were collected. They were sealed in plastic bags, put on iced chest, and transferred to the Crop Physiology Laboratory, Hellenic Sugar Industry SA, Larissa factory, for leaf dimension measurements. Using *WinDias* image analysis system (*Delta-T Devices*, Cambridge, UK), leaf dimensions were determined in three leaves for each plot. The leaf shape parameters determined using the image analysis system were as follows: leaf area (LA), centroid X or Y (CX or CY), maximum length (L), maximum width (W), average radial (AR), elongation (EL), and shape factor (SF) (see Appendix).

Table 1. Mean monthly temperature and monthly rainfall during the growing season (March to October). Below each column, season mean temperature and season total rainfall are given in bold.

| Month | Mean temperature [ºC] 2003 | 2005 | 2006 | Rainfall [mm] 2003 | 2005 | 2006 |
|---|---|---|---|---|---|---|
| March | 7.3 | 9.3 | 9.8 | 20.1 | 64.2 | 34.1 |
| April | 11.8 | 13.7 | 14.2 | 26.9 | 5.7 | 35.4 |
| May | 20.4 | 20.1 | 19.1 | 47.9 | 16.4 | 1.9 |
| June | 25.3 | 23.5 | 23.8 | 33.2 | 3.6 | 15.3 |
| July | 26.8 | 26.9 | 25.0 | 2.8 | 11.3 | 34.3 |
| August | 26.3 | 25.8 | 26.8 | 5.3 | 24.4 | 10.4 |
| September | 20.2 | 21.8 | 20.7 | 22.8 | 53.4 | 108.3 |
| October | 16.6 | 15.4 | 16.2 | 86.1 | 10.2 | 106.1 |
| | **19.3** | **19.6** | **19.5** | **245.1** | **189.2** | **345.8** |

Data were analyzed as a Randomized Complete Block design combined over years with sampling dates as main factor. Means were compared with LSD test. The *M-STAT* statistical package (version *1.41*, Crop and Soil Sciences Department, Michigan State University) was used for the analysis. Figures were displayed using *Excel 98* software (*MSOffice, Microsoft*) and the significance level of the best-fitted curves was determined by *SPSS 14*.

## Results

**Seasonal changes of LAI and leaf shape parameters:** Years, samplings, and their interaction affected significantly LAI and leaf shape. Only years on CY and year×sampling on EL had no significant effects (Table 2).

LAI was significantly differentiated between years being highest in 2006 (5.60) and lowest in 2003 (1.92). In 2005, mean LAI was high (5.09) but significantly lower than in 2006. LAI was maximized between mid-June and early July and then a decreasing trend was evident (Fig. 1). Analogous to LAI trends were found for LA, L,

Table 2. Analysis of variance (ANOVA) of Leaf Area Index (LAI) and leaf dimension parameters. LA – leaf area, CY – centroid Y, CX – centroid X, L – maximum length, W – maximum width, AR – average radial, EL – elongation, SF – shape factor, and CV – coefficient of variation.

| Source of variation | df | LAI | LA | CY | CX | L | W | AR | EL | SF |
|---|---|---|---|---|---|---|---|---|---|---|
| Blocks | 5 | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Years (Y) | 1 | *** | *** | ns | *** | *** | *** | *** | ** | *** |
| Samplings (S) | 10 | *** | *** | *** | *** | *** | *** | *** | * | *** |
| Y × S | 10 | *** | *** | *** | *** | *** | *** | *** | ns | *** |
| CV [%] | | 25.04 | 11.17 | 5.36 | 4.15 | 6.01 | 7.04 | 5.92 | 6.86 | 5.46 |

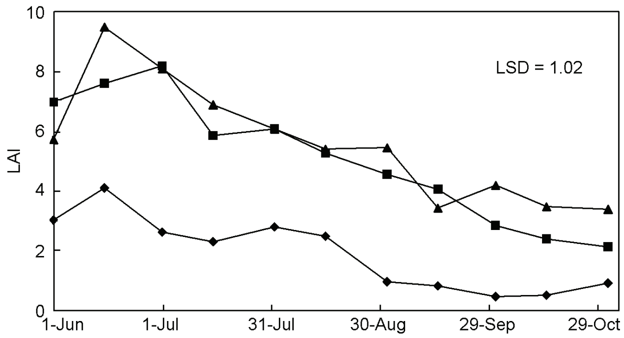


Fig. 1. Seasonal changes of LAI for the three years of experiments.

and moderate ones in 2005. A gradual decrease was evident from early July onward (Fig. 2). No clear seasonal pattern of CY and CX was found. While no significant difference between years was found for CY, CX in 2003 (16.12 cm) was higher compared to 2005 and 2006 (15.26 and 15.11 cm, respectively). A decreasing trend with time was found for EL. This parameter was higher in 2005 (0.633) compared to 2003 and 2006 (0.612 and 0.610, respectively). The highest SF was found in 2003 (2.11), the lowest one in 2005 (1.84), while moderate values (1.90) were measured in 2006. Although SF seasonal patterns in 2005 and 2006 were similar, increasing gradually with time, a completely different pattern was found in 2003. However, in all years SF did not differ significantly from early September onward.

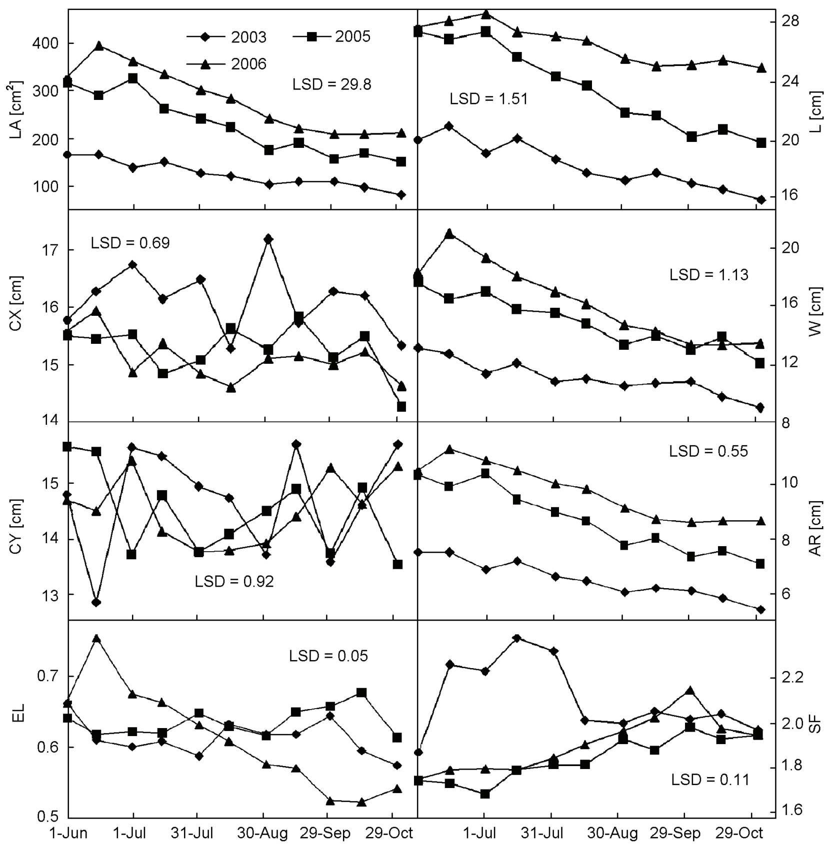


Fig. 2. Seasonal changes of leaf shape parameters. For abbreviations see Table 1.

**Relationships between LA and leaf shape**: Fig. 3 presents the significant relationships between LA and leaf shape parameters. L and W were good predictors of LA since highly significant relationships between LA and leaf dimensions were established. A linear relationship was found between W and LA (y = 28.693 x – 192.33, $r^2$ = 0.97, $p$<0.001). The strongest relationship was that between AR and LA, which was identical (y = 4.1192 $x^2$ – 17.628 x + 62.734, $r^2$ = 1.00, $p$<0.001). A quadratic function was the best-fitted curve of the relationship between SF and LA. Correlation coefficient of this relationship was the lowest determined.

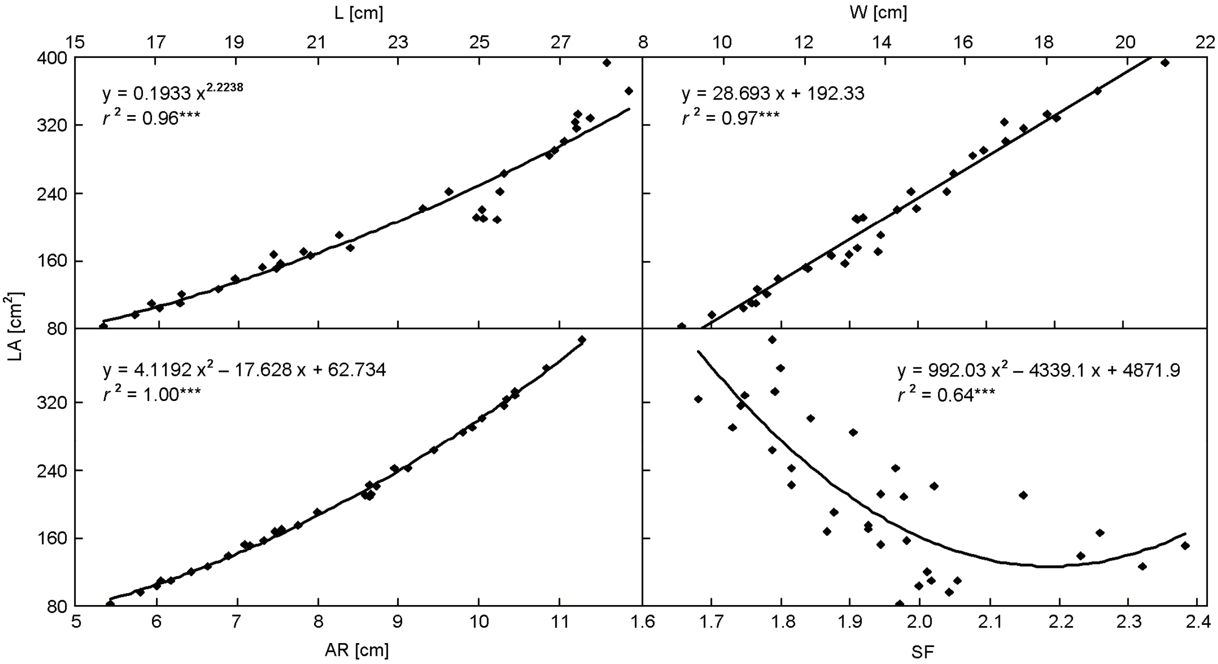


Fig. 3. Best fitted curves of the significant relationships between LA and leaf shape parameters. $^{***}p$<0.001.

**Relationships between LAI and leaf shape parameters**: Quadratic functions described better the relationships between leaf shape parameters (LA, L, W, AR, EL, SF) and LAI (Fig. 4). A highly significant function (y = –0.00001 $x^2$ + 0.0327 x – 2.0413, $r^2$ = 0.90, $p$<0.001) between LA and LAI could be used as a reliable predictor of LAI. Also, a highly significant, best-fitted curve was found between AR and LAI (y = 0.0612 $x^2$ + 0.3544 x – 3.1206, $r^2$ = 0.90, $p$<0.001). Leaf dimensions (L and W) gave highly significant relationships with LAI but had lower $r^2$ (0.82 and 0.88, respectively) compared to LA and AR (Fig. 4). Even lower $r^2$ was found for the quadratic relationship between SF and LAI. A significant but weak curvilinear relationship was evident between EL and LAI (y = 183.14 $x^2$ – 205.92 x + 61.172, $r^2$ = 0.22, $p$<0.05).

## Discussion

The aim of this work was to study the heteroblasty of sugar beet during growing season and to relate leaf shape changes with LAI. Sugar beet leaves showed high plasticity to environmental conditions since all leaf shape parameters were affected by year and sampling time. With the progress of the growing season, leaves tended to become smaller (LA, L, W, and AR were decreased) and rounded as it was indicated by the changes in EL and SF (Fig. 2). The largest leaves were found in 2006.

Confirming previous results, LA could be precisely determined by measuring leaf dimensions (Tsialtas and Maslaris 2005). This is of high importance especially under field conditions where easy, non-destructive determination of LA based on simple functions is needed (Lu *et al.* 2004). Thus, the linear, highly significant relationship between W and LA could be helpful. Such functions could be extremely useful when successive LA determinations are needed as in relative leaf expansion rate determination, which is related with sugar beet tolerance to drought (Ober and Luterbacher 2002, Ober *et al.* 2005). The identical relationship between AR and LA could not be used due to difficulties in estimating this parameter especially in the field. In dicotyledonous species, shape or size formation of determinate organs, such as leaves, is a function of cell division and cell elongation occurring simultaneously throughout leaf expansion (Tsukaya 2003). In our case, increases in LA were the result of leaf elongation as it derived from the negative relationship between LA and SF.

LAI, after reaching its maximum at the end of June

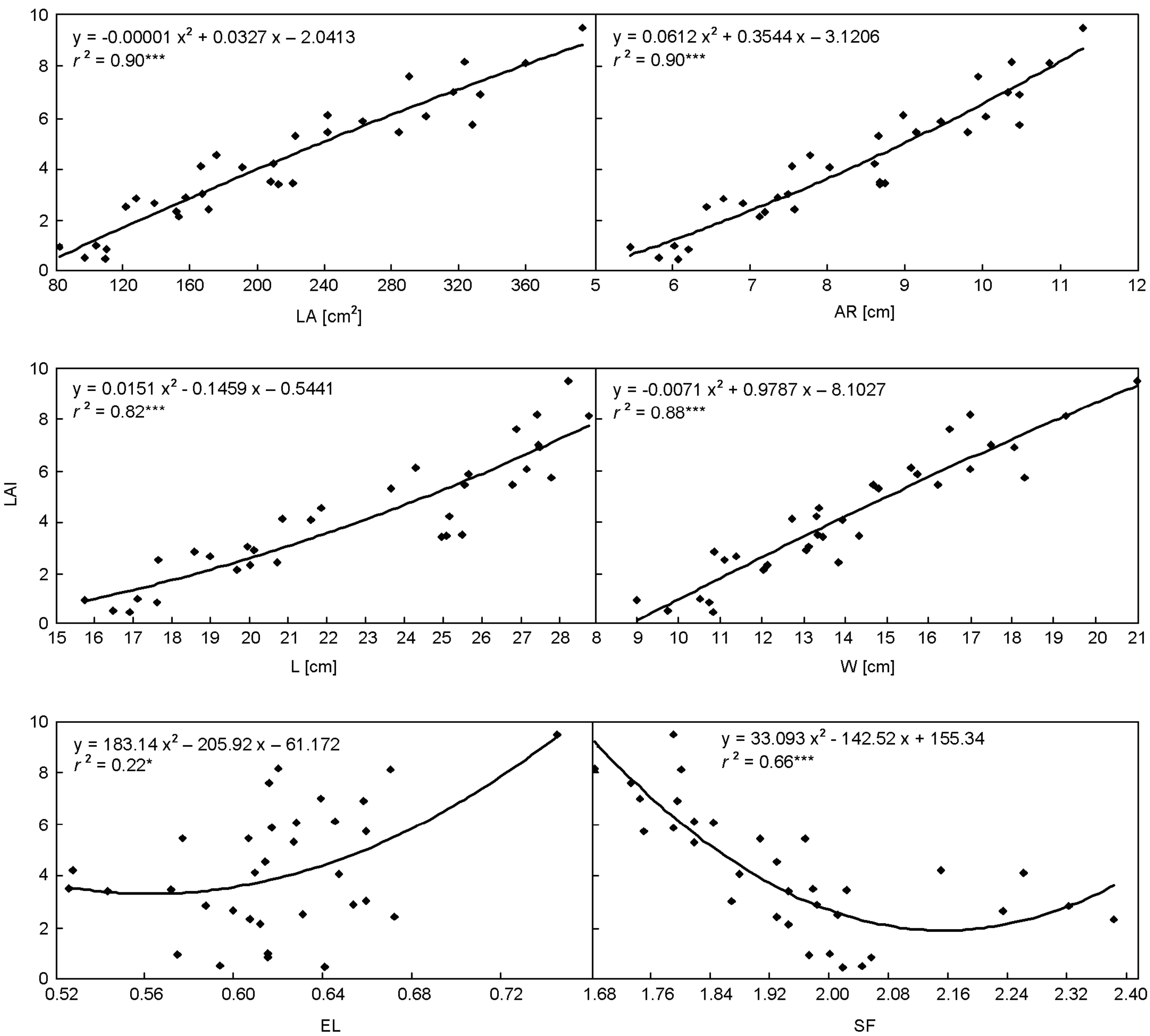


Fig. 4. Best fitted curves of the significant relationships between LAI and leaf shape parameters. $^{*}p<0.05$, $^{***}p<0.001$.

or the beginning of July, declined gradually toward the end of season. It was quite plastic since it was significantly different between years. LAI is strongly related with radiation interception by crop and an optimum of *ca*. 3.5–4.0 was defined for sugar beet in order to maximize radiation absorption (Scott and Jaggard 1993). For this reason, LAI is an important parameter incorporated in yield prediction models (Launay and Guérif 2003) and its easy determination, especially in the field, is necessary. Non-destructive, instrumental methods for LAI estimation have been evolved and are widely used in field (Jonckheere *et al.* 2004, Weiss *et al.* 2004). In sugar beet, instrumentally determined LAI is strongly related with the destructively determined one (Röver and Koch 1995). However, destructive determination is laborious and time-consuming while instrumental methods are based on expensive equipment. Thus, easy, non-destructive estimation of LAI based on leaf dimension parameters is of high importance. Analogous estimations were established for common bean by estimating the LA of the central leaf (de Jesus *et al.* 2001) or for cucumber and tomato by measuring leaf L and W (Blanco and Folegatti 2003). In our case, highly significant quadratic functions between LA or leaf dimensions (L, W) and instrumentally determined LAI were evident. These functions could be easily used for non-destructive LAI determination of the Rizor cultivar. Although LAI was significantly related with AR, this function is of no use due to the difficulty of the independent parameter determination under field conditions. As it is derived by the relationships between LAI and SF, high LAI values are related with more elongated leaves.

Since LAI decreased with time progress, probably as a result of the abiotic stress effects (*e.g.* drought), smaller and more rounded leaves lowered LAI values.

Concluding, leaf shape changes during growing season were related with LAI in sugar beet cv. Rizor. Easily applied functions for non-destructive LA and LAI determination were found for use in field.

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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 center 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

$$SF = \frac{P}{P_c}$$

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