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Non-destructive models for leaf area determination in canola

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Key words:

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ABSTRACT

The leaf is a very important structure of the plants, since it allows gas exchanges and the transformation of light energy into chemical energy. This study aimed to generate and test mathematical models for leaf area estimation in canola based on leaf dimensions. Two experiments were conducted with canola in 2014, in which leaves were collected in different phenological stages with different sizes and shapes. Subsequently, leaf length, width and area were measured (with automatic meter) in 606 leaves, which included 371 ovate and 235 lanceolate leaves. The models were generated using length, width and length versus width as independent variables and leaf area as dependent variable. The models were validated using a group of leaves different from those used to generate the models. A total of 27 models were obtained and those with best statistics and higher simplicity were selected. The polynomial model $LA = 0.88735 W^2 + 0.93503 W$ and the power model $LA = 1.1282 W^{1.9396}$ can be used for both types of leaves and have high accuracy in the estimation of canola leaf area.

Palavras-chave:

Brassica napus
folhas ovaladas
folhas lanceoladas
modelagem

Modelos não destrutivos para determinação da área foliar em canola

RESUMO

A folha é uma estrutura de grande importância para as plantas, visto que por meio dela ocorrem as trocas gasosas e a transformação da energia luminosa em energia química. O objetivo neste trabalho foi gerar e testar modelos matemáticos para a estimativa de área foliar de folhas de canola em função das dimensões foliares. Para isto foram realizados dois experimentos com canola no ano de 2014 e coletadas folhas em diferentes estágios fenológicos e de diferentes tamanhos e formas; posteriormente foram medidos o comprimento, a largura e a área foliar (com medidor automático) de 606 folhas dentre as quais 371 ovaladas e 235 lanceoladas. Os modelos foram gerados utilizando-se comprimento, largura e comprimento versus largura como variáveis independentes e a área foliar como variável dependente. Os modelos foram validados com um grupo distinto de folhas dos utilizados para a geração. Obteve-se o total de 27 modelos escolhendo-se aqueles que apresentaram as melhores estatísticas e maior simplicidade. Os modelos que podem ser utilizados para ambos os tipos de folhas e apresentam alta precisão de estimativa de área foliar em canola, são o modelo polinomial $AF = 0,88735 L^2 + 0,93503 L$ e o modelo potencial $AF = 1,1282 L^{1,9396}$.



INTRODUCTION

Canola (*Brassica napus* L. var. *oleifera*) is a cold-season oilseed plant cultivated in Southern Brazil (Krüger, 2011). Its yield is influenced by different environmental, physiological and morphological factors, among which leaf area (LA) is a parameter indicative of crop yield, since biomass accumulation occurs through photosynthesis and the leaf is the main plant organ, responsible for gas exchanges and light energy interception (Pereira et al., 1997; Favarin et al., 2002).

Accurate LA measurements are essential to understand the interaction between plant growth and the environment in which they develop (Jesus et al., 2001). There are non-destructive methods for LA determination using simple and easily operated devices that preserve leaf integrity (Adami et al., 2008; Fagundes et al., 2009; Bakhshandeh et al., 2011). Some examples are models generated for tomato and cucumber (Blanco & Folegatti, 2003), melon (Lopes et al., 2007), sunflower (Maldaner et al., 2009) and crambe (Toebe et al., 2010).

For the canola crop, there are the models of Chavarria et al. (2011) and Cargnelutti Filho et al. (2015). However, applying only one model to determine canola leaf area may not be adequate, because the leaves are morphologically different along its development (Iriarte & Valetti, 2008) and one model for each cultivar becomes unviable, since new cultivars are frequently released. On the other hand, it is necessary to verify which is the variability existing between cultivars and cultivation environments for canola leaf area in the producing regions of Brazil. Thus, this study aimed to generate and test mathematical models for leaf area estimation in canola based on leaf dimensions.

MATERIAL AND METHODS

The experiment was carried out at the Department of Plant Science of the Federal University of Santa Maria (UFSM), in the municipality of Santa Maria, RS, Brazil (29° 43' 23" S; 53° 43' 15" W; 95 m). The climate of the region, according to Köppen's classification, is Cfa, humid subtropical with hot summers and without a defined dry season (Heldwein et al., 2009). The soil in the experimental area is classified as sandy dystrophic Red Argisol (Streck et al., 2008). Soil correction and fertilization were based on its chemical analysis, following the recommendations of the manual of fertilization and liming for the states of Rio Grande do Sul and Santa Catarina (SBCS, 2004).

The spacing used in the first experiment was 0.30 m between rows and 0.08 m between plants, totaling a population of 416,666 plants ha⁻¹; the second experiment used a spacing of 0.50 m between rows and 0.06 m between plants, totaling a population of 400,000 plants ha⁻¹. The first experiment was sown on May 15, 2014, and emergence occurred on May 20, 2014; the second experiment was sown on June 9, 2014, and emergence occurred on June 15, 2014. The experiments were set in randomized blocks, in a split-plot scheme, with four replicates. Three manual weeding were performed for weed control, while pests (*Diabrotica speciosa* L.) were controlled through the application of insecticide in the vegetative stage.

Four canola cultivars were sown: Hyola 433, Hyola 411, Hyola 420 and Hyola 61. Three leaf collections were performed in these experiments, on July 28, July 29 and August 13, 2014, which encompassed the stages of formation of rosette, flower bud and flowering. Samples were collected in different phenological stages and in leaves of different sizes and shapes, because canola plants produce leaves of different shapes along the cycle.

These leaves with different shapes can be grouped into two distinct categories; the first one is called ovate and includes basal and petiolate leaves, arranged in the form of a rosette, while the second one, the lanceolate, refers to smaller leaves and amplexicaul leaves that emerge after stem elongation.

After plant collection, the leaves were separated from the stem and only those photosynthetically active, with no damage or deformation caused by diseases, insects or other external factors, were selected. Length, width and area were measured in these leaves, in a total of 606 leaves, 371 ovate and 235 lanceolate, with width from 0.3 to 16.5 cm, length from 1.8 to 22.5 cm and leaf area from 0.36 to 273.97 cm².

Length (L) was measured with a graduated ruler, considering the length of leaf blade along the midrib, disregarding the petiole. Width (W) was determined by measuring the longest width of the leaves perpendicularly to the midrib. Leaf area (LA) for both types of leaves was obtained with a leaf area integrator (LICOR 3000).

The models were generated using length (L), width (W) and the product of length versus width (L.W) as independent variables, and the measured LA as dependent variable. Linear, quadratic, exponential and power models were obtained using the program Sigma Plot®. Models with distinction between ovate and lanceolate leaves were generated using 186 ovate leaves and 117 lanceolate leaves, while models without such distinction were generated using 303 mixed leaves. The aim was to identify which independent variable is best correlated with LA and whether a general model can be used to determine canola LA or if it is necessary to use one model for each type of leaf. The slope was estimated by forcing the line to pass through the origin (null intercept), because it is more correct according to Richter et al. (2014), since if there are no linear dimensions, there is no leaf area.

For validation, the obtained models were tested with a set of leaves different from those used to generate the models, but with the same number of mixed, ovate and lanceolate leaves. LA estimated by the mathematical model was compared with the LA measured through the analysis of data dispersion around the 1:1 line, in order to select the model in which the line generated between the values observed and obtained by the model was as close as possible to the 1:1 line.

The best model for the estimation of canola leaf area, as a function of L (cm), W (cm) and/or L.W (cm²), was selected based on the statistics of coefficient of determination (R²), Akaike information criterion (AIC) (Motulsky & Christopoulos, 2003; Floriano et al., 2006), Willmott's index of agreement (d) (Willmott et al., 1985) and BIAS index (Leite & Lima, 2002). The higher the values of R² and d, the lower the values of root-mean-square error (RMSE) and AIC, and the closer the BIAS index is to zero, the better the model is. The

model developed by Chavarria et al. (2011) in Passo Fundo, RS, was also tested, in order to verify whether this model was valid for both types of canola leaves.

RESULTS AND DISCUSSION

Initially, the relationship of length, width and length versus width (L.W) with leaf area was tested through linear, quadratic, power and exponential regressions. However, the exponential equations did not fit and were disregarded from the study. After this previous analysis, 27 linear, quadratic and power models were obtained (Table 1). Isolated models for each cultivar were also generated, but the coefficients of each model were similar to those of general models. Thus, there was no need for one model for each cultivar, which is desired, because new cultivars are released every year and it can disqualify the model for future use.

Canola leaf area is estimated with greater accuracy by the models that use the product of leaf Length x Width (L.W) as independent variable, compared with models that use only one leaf dimension (Table 1). Similar results were obtained by Hinnah et al. (2014), Serdar & Demirsoy (2006) and Keramatlou et al. (2015) for eggplant, chestnut and Persian walnut, respectively. This result occurs because the product

L.W is an area, thus requiring the adjustment of the reducing coefficients in each model.

For mixed leaves, LA is best estimated by polynomial and power equations, Models 8 and 9, respectively, which use L.W as independent variable. However, the Models 1 and 2, which use only width (W) as independent variable, show similar results, with the advantage of using only one variable, thus surpassing the models for ovate leaves when only W is used (Table 1).

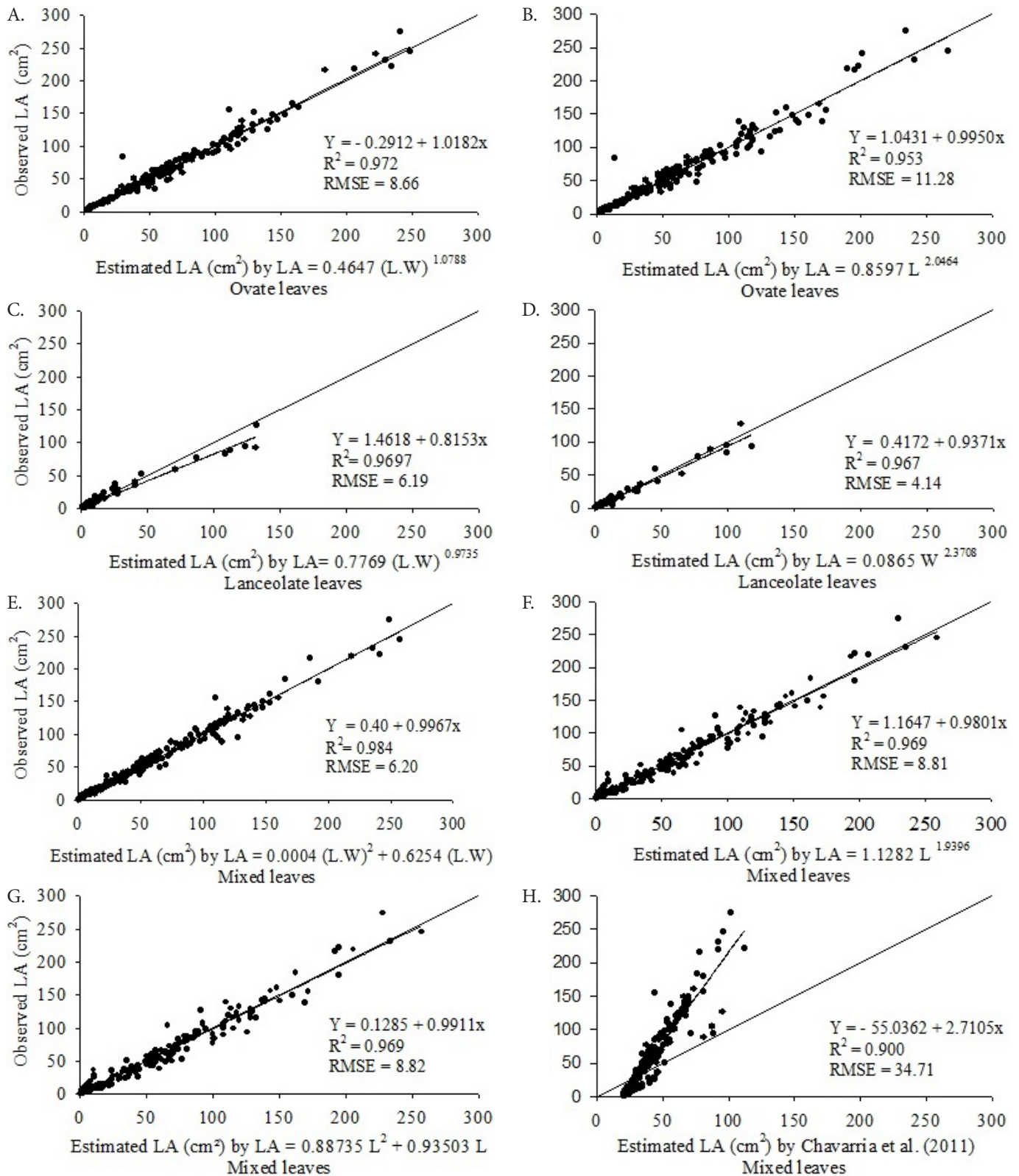
LA is best estimated by the power equation, Model 19, for ovate leaves and by the power equation, Model 29, for lanceolate leaves. Both use L.W as independent variable (Table 1). When the choice is only one of the leaf dimensions to estimate leaf area, the independent variable with highest correlation with LA varies depending on the type of leaf. For lanceolate leaves, length (L) is the best variable to estimate LA, while width (W) is the best variable for ovate leaves. Thus, the power equations Model 12 for ovate leaves, which requires only the variable W, and Model 25 for lanceolate leaves, which requires only the variable L, show good results, with the advantage of using only one of the leaf dimensions.

The analysis of data dispersion around the 1:1 line indicates that some of the developed models show high capacity and accuracy for the estimation of canola leaf area (Figure 1).

Initially, due to the morphological difference between the leaves, the use of different models to accurately estimate canola

Table 1. Regression models for the estimation of canola leaf area (LA, cm²) with the respective coefficients of determination (R²) of their generation and the Akaike information criterion (AIC), root-mean-square error (RMSE), Willmott's index of agreement (d) and BIAS index of their test, as a function of length (L), width (W) and the product of length versus width (L.W) using mixed, ovate and lanceolate leaves

Model	Equation	R ²	Test of the models			
			AIC	RMSE	d	BIAS
Mixed leaves						
1) Polynomial	LA = 0.88735 W ² + 0.93503 W	0.94	1464.3	8.82	0.928	0.0059
2) Power	LA = 1.1282 W ^{1.9396}	0.94	1469.9	8.81	0.928	-0.0075
3) Linear	LA = 9.29440 W	0.81	1815.3	21.11	0.790	0.1271
4) Polynomial	LA = 0.47489 L ² - 0.70612 L	0.86	1729.5	16.83	0.865	-0.0653
5) Power	LA = 0.33393 L ^{2.08983}	0.85	1732.2	16.98	0.856	-0.0498
6) Linear	LA = 5.5748 L	0.66	1996.7	28.95	0.502	0.1310
7) Chavarria et al. (2011)	LA = 0.2023L ² - 0.4267 L + 20.148	0.77	-	34.71	0.235	-0.1561
8) Polynomial	LA = 0.0004 (L.W) ² + 0.6254 (L.W)	0.97	1177.0	6.20	0.957	-0.0061
9) Power	LA = 0.4888 (L.W) ^{1.0677}	0.97	1187.9	6.36	0.956	-0.0180
10) Linear	LA = 0.689 (L.W)	0.97	1210.8	6.96	0.952	0.0044
Ovate leaves						
11) Polynomial	LA = 1.00032 W ² - 0.42101 W	0.95	866.0	11.32	0.905	-0.0129
12) Power	LA = 0.8597 W ^{2.0464}	0.95	866.1	11.28	0.906	-0.0114
13) Linear	LA = 9.34484 W	0.76	1193.2	26.78	0.588	0.0965
14) Polynomial	LA = 0.50378 L ² - 0.31360 L	0.94	932.4	14.13	0.881	-0.0102
15) Power	LA = 0.4484 L ^{2.0270}	0.94	933.2	14.22	0.878	-0.0055
16) Linear	LA = 0.3364 L ^{2.1296}	0.96	1210.1	29.28	0.520	0.1035
17) Chavarria et al. (2011)	LA = 0.2023L ² - 0.4267 L + 20.148	0.77	-	72.27	0.431	-0.334
18) Polynomial	LA = 0.00034 (L.W) ² + 0.63338 (L.W)	0.98	670.2	8.58	0.932	-0.0082
19) Power	LA = 0.4647 (L.W) ^{1.0788}	0.98	666.8	8.66	0.932	-0.0133
20) Linear	LA = 0.69489 (L.W)	0.98	705.2	9.55	0.921	0.0097
Lanceolate leaves						
21) Polynomial	LA = 1.29823 W ² + 2.75466 W	0.92	340.1	9.26	0.854	0.1267
22) Power	LA = 3.4835 W ^{1.6235}	0.92	339.5	8.05	0.868	0.0776
23) Linear	LA = 8.09020 W	0.77	461.2	7.76	0.708	0.1627
24) Polynomial	LA = 0.28005 L ² - 0.63213L	0.96	249.9	4.00	0.925	0.0164
25) Power	LA = 0.0865 L ^{2.3708}	0.96	234.3	4.14	0.930	0.0303
26) Linear	LA = 2.17945 L	0.58	530.7	13.71	0.328	0.1498
27) Chavarria et al. (2011)	LA = 0.2023L ² - 0.4267 L + 20.148	0.77	-	18.35	0.475	1.4127
28) Polynomial	LA = - 0.00029 (L.W) ² + 0.72027 (L.W)	0.97	179.8	9.58	0.888	0.1554
29) Power	LA = 0.7769 (L.W) ^{0.9735}	0.97	180.1	6.19	0.912	0.0786
30) Linear	LA = 0.69276 (L.W)	0.97	181.1	6.74	0.907	0.0688



The transverse line in each figure is the 1:1 line

Figure 1. Observed leaf area versus leaf area estimated using width (W), length (L) and length versus width (L.W) of canola leaves through various developed equations and the equation of Chavarria et al. (2011)

leaf area was expected to be necessary. However, according to the tests of the models, it is clearly possible to use one general model (with no difference regarding the type of leaf), since the RMSE of the model that uses L.W or only width (W) is lower than 8.82 cm², below the values obtained by Kumar (2009), Toebe et al. (2012) and Hinnah et al. (2014), who found minimum RMSE in their models of 71.79 for saffron, 12.56 for

snap bean and 33.2 for eggplant, respectively. However, for researchers who need greater accuracy in leaf area estimation, the models can be separated according to the stage of the plant, since in the vegetative stage (until the beginning of flowering) the plant shows more ovate leaves. If leaf area needs to be determined in this stage, the accuracy using the model for ovate leaves will be higher.

The models selected to estimate leaf area in lanceolate and ovate leaves showed good results, with low RMSE, which varied from 4.14 to 11.28 cm² (Figure 1A; 1B; 1C and 1D), although the models proposed for lanceolate leaves slightly underestimated leaf area. Although these models have high accuracy, their use is more time-consuming, because it is necessary to collect data of leaf length and width, besides classifying leaves as ovate and lanceolate.

Therefore, the general model that uses L.W as independent variable, proposed for all types of leaves, showed excellent results, with data well distributed around the 1:1 line and RMSE of only 6.20 cm² (Figure 1E). Polynomial and power models, both using only width (W) as independent variable, showed high capacity to estimate canola leaf area, with data well distributed around the 1:1 line and RMSE of only 8.81 and 8.82 cm², respectively (Figure 1F and 1G), and with the advantage of using only one of the leaf dimensions, which reduces by 50% the number of measurements, decreasing the working time at the field. The measurement of only one variable is preferable, because, according to Kumar (2009) and Floriano et al. (2006), one must opt for the simplicity and convenience of the models used, provided that they show good fits to the data. These selected models also have lower AIC, d close to 1, and BIAS, close to zero.

The model proposed by Chavarria et al. (2011) was not able to estimate canola leaf area accurately, because it initially underestimates and then overestimates the values, without following the 1:1 distribution (Figure 1H). Its application resulted in the highest RMSE among the tested models (34.7, 72.27 and 18.35 cm²), which may have occurred because the authors did not force the equation to pass through the origin (null intercept), because the model estimates a leaf area of 20.148 cm² even when the independent variable (length) is equal to zero. According to Richter et al. (2014), it is important to force the linear regression to pass through the origin (null intercept), because if there are no linear dimensions, there must not be leaf area. Additionally, Chavarria et al. (2011) also used leaf length (L) as independent variable. However, the variable with highest correlation with canola leaf area when a general model is used is leaf width (W), which is corroborated by the results of Cargnelutti Filho et al. (2015).

The coefficients of the models obtained in the present study for four different cultivars were close to those obtained by Cargnelutti Filho et al. (2015) individually for three cultivars, indicating that one single model can be used for the different canola cultivars under the climatic conditions of Santa Maria-RS.

In tests conducted with the equations of Cargnelutti Filho et al. (2015) for Hyola 61 and Hyola 433, with the equations specific for the LA of these cultivars, there was underestimation of more than 10% in the estimated values of LA compared with those in the present study. On the other hand, the general power equation (Model 2) showed deviations in the 1:1 tendency of at most 4% when the cultivars were separated, which demonstrate the capacity for LA estimation of mixed leaves of the power model developed in the present study.

CONCLUSIONS

1. Canola leaf area can be accurately estimated based on linear dimensions in a non-destructive way.

2. The morphological difference of the leaves does not have high influence on leaf area estimation, provided that the model based on length versus width (L.W) or only width (W) is used.

3. The polynomial model $LA = 0.88735 W^2 + 0.93503 W$ and the power model $LA = 1.1282 W^{1.9396}$ can be used for both types of leaves and have high accuracy in the estimation of canola leaf area.

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