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A non-destructive method for estimating onion leaf area

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Abstract

Leaf area is one of the most important parameters for characterizing crop growth and development, and its measurement is useful for examining the effects of agronomic management on crop production. It is related to interception of radiation, photosynthesis, biomass accumulation, transpiration and gas exchange in crop canopies. Several direct and indirect methods have been developed for determining leaf area. The aim of this study is to develop an indirect method, based on the use of a mathematical model, to compute leaf area in an onion crop using non-destructive measurements with the condition that the model must be practical and useful as a Decision Support System tool to improve crop management. A field experiment was conducted in a 4.75 ha commercial onion plot irrigated with a centre pivot system in Aguas Nuevas (Albacete, Spain), during the 2010 irrigation season. To determine onion crop leaf area in the laboratory, the crop was sampled on four occasions between 15 June and 15 September. At each sampling event, eight experimental plots of 1 m^2 were used and the leaf area for individual leaves was computed using two indirect methods, one based on the use of an automated infrared imaging system, LI-COR-3100C, and the other using a digital scanner EPSON GT-8000, obtaining several images that were processed using Image J v 1.43 software. A total of 1146 leaves were used. Before measuring the leaf area, 25 parameters related to leaf length and width were determined for each leaf. The combined application of principal components analysis and cluster analysis for grouping leaf parameters was used to reduce the number of variables from 25 to 12. The parameter derived from the product of the total leaf length (L) and the leaf diameter at a distance of 25% of the total leaf length (A25) gave the best results for estimating leaf area using a simple linear regression model. The model obtained was useful for computing leaf area using a non-destructive method.

Keywords

leaf area • onion • non-destructive method • mathematical model.

Introduction

Leaf area is one of the most important parameters for characterizing crop growth and development, and is useful for examining the effects of agronomic management on crop production (Hooker 1907; Azzi 1959; Gallagher and Biscoe 1978). This parameter is related to interception of radiation, photosynthesis, biomass accumulation, transpiration and gas exchange in crop canopies (Kucharik *et al*. 1998). It is also one of the most relevant parameters in experimentation, and has been used to predict harvest date (Hammer *et al*. 1995; Kiniry *et al*. 1996). Variables that are useful in agriculture and other disciplines, such as the leaf area index (LAI), which is defined as the total one-sided area of leaf tissue per unit ground surface area (Watson, 1947) are also computed from the leaf area. Accurate measurements of leaf area are essential for understanding the interaction between crop growth and environment (De Jesus *et al*. 2001).

Many methods of leaf area measurement have been developed for several crops (Gower *et al.* 1999; Kussner and Mosandl 2000; Jonckheere *et al*. 2004). Marshall (1968) classified methods to determine leaf area as direct and indirect. Direct methods measure all the leaves of the plant. These methods include the use of millimetric paper (Goodall 1947; Winter *et al*. 1956; Bachman and Keller 1965), planimetric (Daughtry 1990; Nyakwende *et al*. 1997) and gravimetric techniques (Frear 1935, Miller 1938) and tracing, blueprinting and photographing, which require instruments, tools and machines such as hand scanners and laser optic devices (Peksen 2007). An alternative method is the use of image analysis, either with a camera (Tarbell and Reid 1991; Baker *et al.* 1996) or an image scanner (Kershaw and Larsen 1992; Yonekawa *et al*. 1996), combined with digitalization of images. In these cases, the processing is time consuming and sometimes is suitable only for small plants with few leaves.

Indirect methods are useful when this equipment is not available or non-destructive measurements are needed, such as in field conditions or where there is low plant density. Regarding indirect methods, the solar radiation intercepted

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method (Gerdel and Salter 1928; Hibbard *et al*. 1937), automated infrared imaging system (Hatfield *et al*. 1985) or the estimation of leaf area based on linear measurements of the leaf (Ackley *et al*. 1958; Ray and Singh 1989; Astegiano *et al*. 2001) are the most common. Indirect methods enable researchers to measure leaf area on the same plants during the growth period and may reduce variability in experiments (Serdar and Demirsoy 2006). Considering all methods, the estimation method from mathematical models involving linear measurements of leaves (Coombs and Hall 1982; Beerling and Fry 1990) is relatively accurate. Measurements can be made without cutting the plants (Kvet and Marshall 1971). Simple and accurate models eliminate the need for expensive leaf area meters or time-consuming methods, as they are easy to apply and are non-destructive.

The aim of this study is to develop a mathematical model to compute leaf area in an onion crop using non-destructive leaf area measurements. This model should be applicable at different crop stages. The model must also be practical, to be used as a Decision Support System tool to improve crop management.

Materials and methods

The case study

The field experiment was conducted on a 4.75 ha commercial onion plot irrigated with a centre pivot system in Aguas Nuevas (Albacete, Spain), during the 2010 irrigation season. The study area was in warm Mediterranean climate (Papadakis 1966). The soil was classified as a Xeric Torriorthent with a loam texture, medium depth (>600 mm) and a composition of 4% coarse sand, 28% fine sand, 44% silt and 24% clay (USDA 2006). The area was characterised by a flat topography and soils had good drainage, with little sign of water erosion.

Onion seed (cultivar "Pandero") (Kumar *et al*. 2007; Sarkar *et al*. 2008; Enciso *et al.* 2009, Jiménez *et al*. 2010) was directly sown (27.7 plants m^{-2}). Irrigation was scheduled using a simplified water balance method within the root area, following the Food and Agriculture Organization methodology (Pereira and Allen 1999). According to this methodology, the total amount of irrigation water applied was close to 4800 m^3 ha^{-1}, with an average of the accumulated uniformity coefficient (CUac) close to 92%. Data from an agrometeorological station (Campbell Scientific Inc., Logan, USA), located 300 m from the plot were used to obtain the 10-year average and the 2010 irrigation season weather data. Weed growth was controlled by pre-emergence treatment with pendimethalin (33%) at a dose of 2 l ha^{-1}. When the crop had between two and four leaves, a second treatment to control weeds was carried out with oxynil (22.5%) at a dose of 1 l ha^{-1}. Disease control was carried out with two treatments of mancozeb (80%) at 100 and 115 days after sowing. The plot was fertilized with nitrogen (230 kg N ha^{-1}), phosphorous (220 kg P_2O_5 ha^{-1}) and potassium (190 kg K_2O ha^{-1}).

Sampling

In order to determine onion crop leaf area in the laboratory during crop development (18 March to 22 September) the crop was sampled on four occasions between 15 June and 15 September. On each occasion, eight experimental sub-plots of 1 m^2 were used, which were selected at random across the plot. Forty plants were collected throughout the plot on each sampling occasion. A total of 1146 leaves were measured across all the sampling occasions.

On each sampling occasion, the leaf area of individual leaves was computed using two indirect methods. The first method was based on the use of an automated infrared imaging system, LI-COR-3100C (LI-COR Inc., Lincoln, Nebraska, USA). The second method used a digital scanner EPSON GT-8000 (Seiko Epson Corporation, Nagano, Japan), producing images that were processed using Image J v 1.43 software.

Before measuring leaf area by the two indirect methods, 25 parameters were determined for each leaf (Table 1). Some of these parameters were previously used to determine the leaf area in onion crops (Hoffman 1971; Gamiely *et al*. 1991) and grapes (Legorburo 2005). Five of the parameters, defined as the main parameters, were measured directly with a tape measure (± 1 mm accuracy): total leaf length (L), leaf width at base (A), leaf width from a distance of 25% (A25), 50% (A50) and 75% (A75) from leaf base. The remaining parameters (a total of 20) were computed using mathematical relationships of the main parameters. The computed parameters were associated with leaf size (L×A, L×A25, L×A50, L×A75, A×A25, A×A50, A×A75, A25×A50, A25×A75, A50×A75) and leaf shape (L/A, L/A25, L/A50, L/A75, A/A25, A/A50, A/A75, A25/A50, A25/A75, A50/A75).

Statistical analysis

Statistical analyses were performed using SPSS software (SPSS, 2008). Descriptive statistics were calculated [average, standard deviation, minimum and maximum value, and coefficient of variation (CV)] to determine the variability of the measured and computed parameters.

Principal components analysis (PCA) was used to represent most of the variance among a large number of variables (in this case the parameters of each leaf) by a much smaller number of variables, termed factors (Pearson, 1902; Hotelling, 1933; Haan, 2002), which are linear combinations that maximize the shared portion of the variance. The objective of PCA is to obtain linear combinations of representative variables that exhibit maximum variance for a multidimensional

Table 1. ***Description of the measured and the computed parameters determined for each leaf.***

Parameter	Description
L (mm)	Total leaf length
A (mm)	Leaf base width
A25 (mm)	Leaf width at a distance of 25% from the leaf base
A50 (mm)	Leaf width at a distance of 50% from the leaf base
A75 (mm)	Leaf width at a distance of 100% from the leaf base
L×A	L and A product
L×A25	L and A25 product
L×A50	L and A50 product
L×A75	L and A75 product
A×A25	A and A25 product
A×A50	A and A50 product
A×A75	A and A75 product
A25×A50	A25 and A50 product
A25×A75	A25 and A75 product
A50×A75	A50 and A75 product
L/A	L and A ratio
L/A25	L and A25 ratio
L/A50	L and A50 ratio
L/A75	L and A75 ratio
A/A25	A and A25 ratio
A/A50	A and A50 ratio
A/A75	A and A75 ratio
A25/A50	A25 and A50 ratio
A25/A75	A25 and A75 ratio
A50/A75	A50 and A75 ratio

phenomenon and are also uncorrelated. Hence, it is possible to determine which parameters are more useful in explaining the leaf characteristics. The following steps were followed:

• Computation of the correlation matrix, to identify the most important correlation structures between leaf parameters and correlation coefficients. The correlation matrix was useful to explain the groups obtained in the next step, cluster analysis (CA) of the parameters.

• Computation of the percentage of variance explained. Thus, the selected factors were those representing a cumulative variance higher than 90%.

• Computation of the rotated component matrix. Rotated loadings were determined for each factor using loading coefficients (Malinowski and Howery, 1980). Loading coefficients of the rotated matrix showed the participation of each leaf parameter in the formation of each factor.

A CA was then applied to the original group of variables to group parameters with similar characteristics thereby further reducing the number of parameters (Peña, 2000). An agglomerative hierarchical CA was applied to group parameters, and the similarity–dissimilarity measure was used for the correlation coefficient (Alhamed *et al*., 2002; Unal *et al*., 2003). Single linkage was used to link clusters, which is based on the shortest distance between objects.

The parameters selected using PCA and CA were used to obtain regression models between those parameters and leaf area. A general linear regression model (GLM) and several simple linear regression models were utilized (models 1 to 4).

Model development

Several models were tested. After confirming that the data met the assumptions of parametric statistical approaches, an analysis of regression residuals, by means of normality, homoscedasticity and independence test, was carried out to validate each model proposed. In addition, to compare the model predictions and the measurements, for each model, the goodness of fit was determined using the regression coefficient (R^2) (Astegiano *et al*., 2001; Cittadini and Peri, 2006). The models used were:

Model 1: y = a x; previously used to estimate leaf area in tomato (Lyon, 1948; Balakrishnan *et al*., 1992), cotton (Ashley *et al*., 1963), rice (Johnson, 1967; Palaniswamy and Gomez, 1974) and maize (McKee, 1964; Giovanardi, 1972).

Model 2: y = a+cx; previously used to estimate the leaf area of grapes (Manivel and Weaver, 1973; Smith and Kliewer, 1983; Elsner and Jubb, 1988), cucumber (Liebig, 1978; Robbins and Pharr, 1987), pepper (Ray and Singh, 1989), tomato (Astegiano *et al*., 2001) and onion (Gamiely *et al*., 1991).

Model 3: log y = a+c logx; previously used in grapes (Sepúlveda and Kliewer, 1983; Elsner and Jubb, 1988; Silvestre and Eiras-Dias, 2001), maize (Tarbell and Reid, 1991), and onion (Hoffman, 1971).

Model 4: logy= a logx; previously used by Legorburo (2005) to determine leaf area in grapes.

Statgraphics Plus® software (Llovet *et al*., 2000) and MatLab® functions were used for the PCA, CA, and regression models.

Results

Climatic characteristics

During the irrigation season, the coldest period was between November and March, with the lowest average temperature in December (Table 2). The hottest period was between June and

August, with monthly average temperature close to 26ºC. The large difference between maximum and minimum temperature should be highlighted, with temperatures ranging from 37.6ºC (July) and −14.4ºC (November). The climatic characteristics during the 2010 irrigation season were very similar to the 10-year average weather data (2000–2010) (Table 2).

The rainfall registered during the 2010 irrigation season was 0.1 mm (July) to 61.3 mm (December). Drought during July and August is the most important characteristic of the area.

Descriptive statistical analysis

The variability of the main parameters measured (L, A, A25, A50, and A75) on each sampling occasion is shown in Table 3. The variability of leaf area obtained using the automated infrared imaging system LI-COR-3100C, which showed similar results to the leaf area obtained using a digital scanner, is shown in Table 3.

For parameter L, the CV values ranged from 28.76% (sampling occasion 3) to 38.01% (sampling occasion 1). The variability of parameter A was very similar to parameter L (27.99% on sampling occasion 4, and 32.76% on sampling occasion 2). For the other parameters measured (A25, A50, and A75), the highest variability was seen in A75, with CV from 27.19% (sampling occasion 1) to 61.02% (sampling occasion 2). The greatest variation was shown for AF (except in sampling occasion 2), reaching CV values of 47.4% (sampling occasion 2) to 61.34% (sampling occasion 1).

The correlation between the measured parameters of leaf size (L, A, A25, A50, and A75) was not very strong. The strongest correlation occurred between L and A25 (r =

Table 2. Climatic data of the area of this study for the 10-year average (from 2000–2010) and the 2010 irrigation season obtained from the agrometeorological station (Campbell Scientific Inc., Logan, USA).

	2000-2010					
	Rainfall (mm)	**Reference evapo-transpiration (mm)**	**Maximum tempera-ture (ºC)**	**Minimum tempera-ture (ºC)**	**Average tempera-ture (ºC)**	**Solar radiation (MJ m^{-2})**
January	22.1	35.2	17.7	−9.5	4.2	8.4
February	24.0	49.0	18.8	−7.0	5.4	11.6
March	34.7	83.7	23.8	−5.5	8.5	16.0
April	49.7	107.3	26.5	−0.8	11.5	20.6
May	42.2	142.1	30.6	1.5	15.7	23.9
June	26.2	190.4	35.8	6.4	21.4	27.0
July	4.9	224.7	37.8	9.2	24.5	28.4
August	9.1	194.6	37.7	8.6	23.8	24.6
September	34.9	124.7	33.2	3.9	19.1	18.9
October	47.3	76.4	28.6	0.7	14.1	13.1
November	30.2	40.7	21.1	−5.1	7.9	8.8
December	26.2	29.4	16.4	−8.0	4.7	7.4
	2010					
	Rainfall (mm)	**Reference evapo-transpiration (mm)**	**Maximum tempera-ture (ºC)**	**Minimum tempera-ture (ºC)**	**Average tempera-ture (ºC)**	**Solar radiation (MJ m^{-2})**
January	48.8	29.3	16.1	−11.0	4.7	6.7
February	57.5	41.9	18.4	−4.2	6.0	8.8
March	49.3	75.3	20.7	−6.0	7.7	14.7
April	47.1	99.7	27.1	−2.3	11.2	20.1
May	33.5	135.3	31.1	−0.9	13.6	24.2
June	26.5	167.8	33.8	2.7	18.7	24.7
July	0.5	240.2	37.6	12.1	25.7	28.4
August	11.0	190.4	37.3	3.8	23.3	23.4
September	28.2	123.3	32.8	2.8	18.0	19.3
October	35.1	78.2	27.3	−2.5	11.9	14.6
November	29.5	35.9	20.0	−14.4	4.6	9.0
December	61.3	24.6	16.3	−9.6	2.4	6.7

Table 3. Descriptive statistics of the main leaf parameters measured on each sampling occasion.

07/21/2010	L (mm)	A (mm)	A25 (mm)	A 50 (mm)	A75 (mm)	AF (mm²)
Average	292.7	8.7	10.0	8.1	5.3	4195.0
Standard deviation	111.3	2.7	3.4	4.0	1.4	2573.0
Minimum	45.0	3.0	3.0	2.0	2.0	110.4
Maximum	530.0	17.0	19.0	70.0	11.0	11120.0
Coefficient of variation (%)	38.03	30.95	33.86	49.65	27.20	61.33
08/11/2010	L (mm)	A (mm)	A25 (mm)	A 50 (mm)	A75 (mm)	AF (mm²)
Average	328.7	10.7	12.7	9.9	6.4	6323.0
Standard deviation	96.9	3.5	4.0	2.8	3.9	2997.0
Minimum	45.0	3.0	3.0	3.0	1.0	214.6
Maximum	500.0	25.0	22.0	18.0	70.0	13330.0
Coefficient of variation (%)	29.48	32.77	31.70	28.76	61.02	47.40
09/01/2010	L (mm)	A (mm)	A25 (mm)	A 50 (mm)	A75 (mm)	AF (mm²)
Average	360.4	10.9	13.8	11.3	6.9	6697.0
Standard deviation	103.7	3.4	4.1	3.6	2.7	3215.0
Minimum	70.0	3.0	3.0	2.0	1.0	527.0
Maximum	650.0	26.0	25.0	22.0	17.0	15070.0
Coefficient of variation (%)	28.77	31.76	29.96	31.46	39.03	48.01
09/15/2010	L (mm)	A (mm)	A25 (mm)	A 50 (mm)	A75 (mm)	AF (mm²)
Average	298.4	9.5	11.2	9.3	5.8	4310.0
Standard deviation	111.8	2.7	3.4	2.9	1.9	2420.0
Minimum	70.0	3.0	5.0	4.0	3.0	347.7
Maximum	630.0	17.0	20.0	18.0	13.0	12220.0
Coefficient of variation (%)	37.47	27.99	30.04	31.33	32.52	56.15
All data sampling	L (mm)	A (mm)	A25 (mm)	A 50 (mm)	A75 (mm)	AF (mm²)
Average	321.6	9.9	11.9	9.6	6.1	5488.0
Standard deviation	108.7	3.3	4.1	3.7	2.8	3077.0
Minimum	45.0	3.0	3.0	2.0	1.0	110.4
Maximum	650.0	26.0	25.0	70.0	70.0	15070.0
Coefficient of variation (%)	33.80	33.03	34.08	38.06	45.83	56.07

0.71), A and A25 ($r = 0.77$) and A25 and A50 ($r = 0.72$). All of these correlations were highly significant ($p < 0.001$). The parameters L and A25 showed a strong correlation with leaf area, with coefficient values of 0.87 and 0.88, respectively. Thus, these two parameters can be considered the most adequate to explain leaf area variability (Table 4).

Principal component analysis

In the correlation matrix (data not presented in the results) of the 26 parameters (25 measured and computed parameters and leaf area using LI-COR-3100C) used for PCA, the parameter leaf area had strong, positive correlations (r values of 0.8–0.9) with the parameters L, A25, L×A, L×A25, L×A50, and A×A25. The parameters leaf area and L×A25 had the highest correlation coefficient values ($r = 0.96$). All of these correlations were highly significant ($p < 0.001$).

Strong correlations were also observed among the other parameters associated with leaf size (L×A, L×A25, L×A50, L×A75, A×A25, A×A50, A×A75, A25×A50, A25×A75, A50×A75). The most significant correlations were A25×A75 and A50×A75 ($r = 0.94$), A×A75 and A25×A75 ($r=0.93$), and A and A×A25 ($r = 0.93$).

The parameters associated with leaf shape (L/A, L/A25, L/A50, L/A75, A/A25, A/A50, A/A75, A25/A50, A25/A75, A50/

Table 4. Correlation matrix between the main measured parameters of leaf size.

Parameter	L	A	A25	A50	A75	AF
L	1***	-	-	-	-	-
A	0.64***	1***	-	-	-	-
A25	0.71***	0.77***	1***	-	-	-
A50	0.48***	0.53***	0.72***	1***	-	-
A75	0.26***	0.32***	0.45***	0.49***	1***	-
AF	0.87***	0.72***	0.88***	0.63***	0.40***	1***

****** p< 0.001***

A75), had weaker correlations than the leaf size parameters (L, A, A25, A50, A75, L×A, L×A25, L×A50, L×A75, A×A25, A×A50, A×A75, A25×A50, A25×A75, A50×A75, leaf area) with correlation coefficients lower than 0.8.

According to the PCA (Table 5), six factors were selected, because they represented a total cumulative variance of 96.17% and they had total eigenvalues greater than 1 (Legorburo, 2005). Factor 1 explained nearly half of the variance (close to 45.71%) and the percentage of variance explained by factors 2, 3, 4, 5 and 6 was less important. The total cumulative variance explained by factors 1, 2 and 3 was 77.42%.

In Table 6, the loading coefficient of the rotated matrix for each one of the 26 parameters (25 measured and computed parameter and leaf area) utilized is shown. In factor 1, the most relevant parameters were L×A, L×A25 and A×A25; A75 was most relevant in factor 2. In factors 3–6, the loading coefficient showed that L/A25, A/A25 and A25/A50 were most relevant.

Table 5. Factors that maximize the shared portion of the variance obtained using the principal component analysis technique. Total and cumulative variance explained by each factor.

	Initial eigenvalues	
Factor	**Total variance**	**% Cumulative variance**
1	11.88	45.71
2	5.60	67.26
3	2.64	77.42
4	2.33	86.40
5	1.50	92.16
6	1.04	96.17
7	0.33	97.43
8	0.18	98.11
.	.	.
.	.	.
.	.	.
.	.	.
.	.	.
26	0	100

Cluster analysis

Two clusters were formed, considering a similarity value close to 0.45 (Fig. 1). Cluster 1 was composed of parameters related to leaf size, which included measured (L, A, A25, A50, A75) and computed parameters (the products of L×A, L×A25, L×A50, L×A75, A×A25, A×A50, A×A75, A25×A50, A25×A75, A50×A75). Inside cluster 1, two groupings have a similarity value close to 0.15. The first grouping was composed of parameters L×A25, leaf area, A25, A, A×A25, A×A50, A25×A50, L×A, A50, L×A50, and L. All of these parameters had a loading coefficient of the rotated matrix obtained in PCA higher than 0.6 in Factor 1 (Table 6). In the second grouping in Cluster 1, parameters A75, A×A75, A25×A75, A50×A75 and L×A75 were included, all of which had a loading coefficient of the rotated matrix higher than 0.7 in Factor 2 (Table 6).

Cluster 2 was formed by the parameters related to leaf shape (L/A, L/A25, L/A50, L/A75, A25/A75, A/A75, A/A25, A/A50, A50/A75, A25/A50). The level of similarity was lower than the similarity of parameters in cluster 1. These parameters did not show high similarity with leaf area.

From the results of PCA and CA, the number of parameters was reduced, considering the groupings obtained between parameters and the loading coefficients of the rotated matrix. Therefore, the initial number of parameters was reduced to 13, which included leaf area and the parameters related to leaf size (L, A, A25, A50, A75, L×A, L×A25, L×A50, L×A75, A×A50, A×A75, A25×A75).

Regression models

Regression models were determined using the 13 parameters selected from the PCA and CA. To use parameters collected using the non-destructive method, the leaf area was considered the dependent variable and the remaining 12 parameters as independent variables. To test the models, the measured leaf area of several plants on each sampling occasion was compared to the leaf area values obtained using the proposed models.

The first model used was a GLM using the 12 parameters (Table 7). A model using six independent variables was significant (L×A, L×A25, L×A50, A×A50, A×A75, A25×A75)

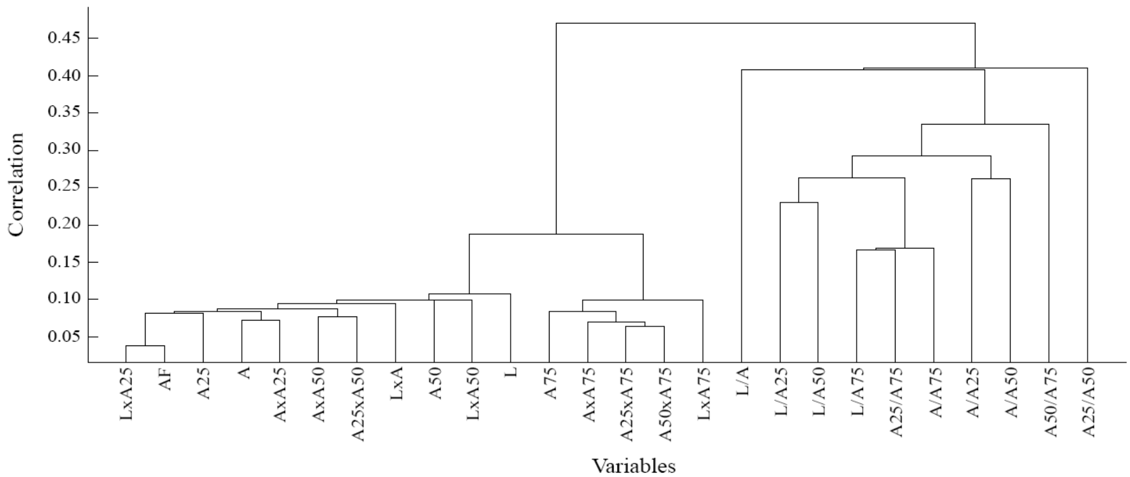

Figure 1. Dendrogram for agglomerative hierarchical cluster analysis using single linkage to group variables used.

Table 6. Loading coefficients of the rotated matrix obtained using the principal component analysis technique, which represent the participation of each leaf parameter in the formation of each factor.

	Factor					
Parameter	**1**	**2**	**3**	**4**	**5**	**6**
L	0.787	0.119	0.562	0.103	−0.075	0.111
A	0.862	0.177	−0.114	0.082	0.391	0.107
A25	0.886	0.280	−0.116	0.117	−0.220	0.173
A50	0.682	0.356	−0.136	0.206	−0.235	−0.466
A75	0.236	0.923	−0.038	−0.234	−0.098	−0.069
L×A	0.913	0.122	0.206	0.089	0.229	0.137
L/A	0.038	0.003	0.824	0.100	−0.512	0.021
L×A25	0.917	0.185	0.184	0.097	−0.164	0.175
L/A25	-0.065	−0.107	0.917	0.055	0.322	−0.104
L×A50	0.869	0.268	0.186	0.143	−0.203	−0.219
L/A50	0.031	−0.107	0.748	0.219	0.322	0.475
L×A75	0.574	0.732	0.241	−0.168	−0.126	0.009
L/A75	0.078	−0.129	0.494	0.763	0.161	0.300
A×A25	0.916	0.220	−0.200	0.096	0.104	0.158
A/A25	−0.153	−0.125	0.078	−0.022	0.954	−0.130
A×A50	0.868	0.312	−0.220	0.145	0.076	−0.206
A/A50	0.008	−0.131	0.117	0.134	0.818	0.477
A×A75	0.622	0.722	−0.153	−0.147	0.126	−0.005
A/A75	0.138	−0.166	0.118	0.726	0.535	0.297
A25×A50	0.827	0.361	−0.192	0.151	−0.218	−0.161
A25/A50	0.181	−0.090	0.054	0.226	0.051	0.909
A25×A75	0.580	0.771	−0.120	−0.118	−0.152	0.026
A25/A75	0.202	−0.142	0.048	0.848	−0.060	0.415
A50×A75	0.427	0.846	−0.117	−0.035	−0.144	−0.229
A50/A75	0.210	−0.144	−0.004	0.878	−0.109	−0.316
AF	0.894	0.219	0.204	0.058	−0.159	0.120

and the other six parameters with significance levels greater than 5% were eliminated. The GLM explained 93.43% of leaf area variability (regression coefficient (R^2)), with a high level of significance ($p < 0.01$) and a standard error close to 7.888. The residuals fitted a normal distribution and showed homoscedastic behaviour. With regard to the GLM, the model overestimated the leaf area on all the sampling occasions analysed, with predicted leaf area values ranging from 10% (sampling occasion 2) to 20% (sampling occasion 4) higher than measured. Although the GLM explained a very high percentage of variance in the leaf area, the model included a lot of independent variables, which could make it difficult to use from a practical point of view.

In order to simplify the results, four simple regression models were examined. In each, one leaf area was considered the dependent variable, while the remaining parameters were taken individually as independent variables.

For model 1, the highest regression coefficient (R^2) value (close to 92.43%) and minimum model standard error (8.46) were obtained using parameter L×A25 as the independent variable (Table 8). The dispersion pattern of residuals did not follow a normal distribution and showed heteroscedastic behaviour. Model 1 results were different depending on sampling occasion. Hence, on sampling occasion 1 (21/7) and 3 (9/1), the model tended to overestimate the leaf area, giving predicted leaf area values 15% higher than those measured. In the case of sampling 2 (8/11), this model underestimated leaf area, with predicted values 10% less than measured. For the last sampling occasion (9/15), the leaf area obtained with model 1 showed high divergence, with predicted leaf area values obtained approximately 25% higher than measured.

Model 2 was a logarithmic transformation for the dependent and independent variables (Table 9). Considering parameters L×A or L×A50, the regression coefficient (R^2) reached values close to 90% and higher, for example with the parameter L×A25 (93.08%). The dispersion pattern of residuals did not follow a normal distribution and showed heteroscedastic behaviour. For model 2, the results obtained were slightly different compared with model.1 In most cases analysed (sampling occasion 1, 2 and 3), this model underestimated the leaf area, with predicted leaf area AF values 30% less than measured. On sampling occasion 4 (9/15), predicted leaf areas were approximately 40% less than measured.

In comparison to the other models, model 3 gave the best results for estimating leaf area (Fig. 2), with regression coefficient (R^2) values that were greater for most of the

Table 7. Coefficients and standard error of the proposed general linear regression model using the six most significant independent variables

Independent variable	Coefficients	Standard error
	$-5.96\ 10^{-5}$	0.525
L×A	−0.189	0.059
L×A25	1.234	0.053
L×A50	0.384	0.040
A×A50	−7.486	1.057
A×A75	28.126	3.805
A25×A75	−15.464	2.828

Table 8. Goodness of fit of the simple regression model 1 (y = a x) proposed to estimate the leaf area (y) depending on the independent variable utilized (x). Analysis of regression residuals (normality, homoscedasticity and independence test).

Independent variable	R^2	Standard error	a	Independence	Normal distribution	Homoscedastic
L	69.73	0.001690	0.0178	NO	NO	NO
A	50.82	0.002157	0.5658	NO	NO	NO
A25	70.67	0.001666	0.4826	NO	NO	NO
A50	39.96	0.002384	0.5659	NO	NO	NO
A75	2.28	0.003041	0.8181	NO	NO	NO
L×A	75.04	0.001537	1.5604	NO	NO	NO
L×A25	92.43	0.000846	1.3141	YES	NO	NO
L×A50	79.17	0.001404	1.6419	YES	NO	YES
L×A75	49.35	0.002189	2.4805	YES	NO	YES
A×A50	50.09	0.002173	50.019	YES	NO	NO
A×A75	28.59	0.002600	77.030	YES	NO	YES
A25×A75	30.56	0.002564	61.698	YES	NO	YES

Table 9. ***Goodness of fit of the simple regression model 2 (log y = a logx) proposed to estimate the leaf area (y) depending on the independent variable utilized (x). Analysis of regression residuals (normality, homoscedasticity and independence test).***

Independent variable	R^2	Standard error	a	Independence	Normal distribution	Homoscedastic
L	10.80	0.517	4.162	NO	NO	NO
A	51.71	0.248	1.169	NO	NO	NO
A25	69.66	0.196	1.215	NO	NO	NO
A50	55.63	0.238	1.159	NO	NO	NO
A75	31.43	0.295	1.054	NO	NO	NO
L×A	85.26	0.137	0.927	NO	NO	NO
L×A25	93.08	0.093	0.954	YES	NO	NO
L×A50	88.73	0.119	0.921	NO	NO	NO
L×A75	77.91	0.167	0.855	NO	NO	NO
A×A50	57.80	0.232	0.582	NO	NO	NO
A×A75	46.95	0.260	0.555	NO	NO	NO
A25×A75	54.59	0.240	0.565	NO	NO	NO

parameters considered (Table 10). The best result was obtained using parameter L×A25, although the variability explained by the model did not improve more than in models 1 and 2. The model selected, leaf area = 0.000199 + 1.277 L×A25, explained 92.53% of the variability of leaf area, and the dispersion pattern of the residuals followed a normal distribution and showed homoscedastic behaviour. Moreover, the model showed good results when used on each sampling occasion. On sampling occasion 1 and 3, predicted leaf area using this model was 8% higher than measured. For sampling occasion 2 and 4, the model underestimated the leaf area, with predicted values approximately 6% less than measured. The variability explained for some parameters by model 4 (Table 11) was slightly higher than in the other models considered. The highest regression coefficient (R^2) value reached was for parameter L×A25 (93.45%), although the dispersion pattern of residuals did not follow a normal distribution and showed heteroscedastic behaviour. Model 4 tended to overestimate leaf area values in all the sampling occasions. Hence, predicted values were close to 28% (sampling occasion 1 and 3), 12% (sampling occasion 3) and 14% (sampling occasion 4) higher than measured.

Discussion

All measured parameters showed high variability on each sampling occasion. This is explained by the intrinsic heterogeneous size of leaves on each onion plant and also by the variability of plant size between the different sampling

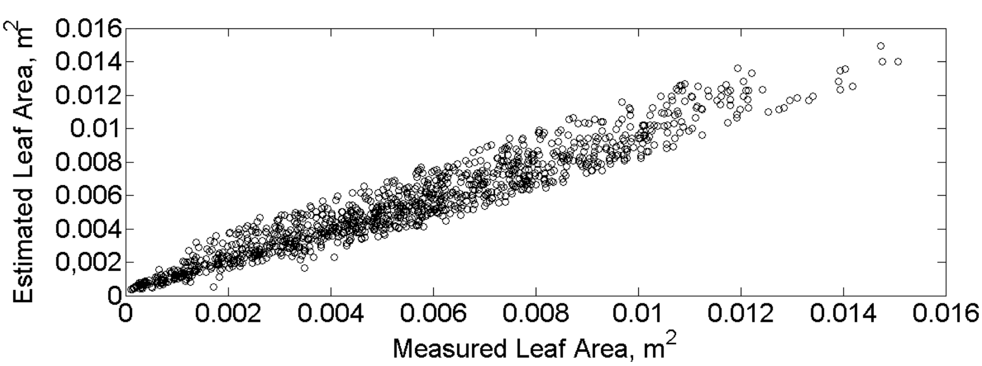

Figure 2. Relationship between the measured leaf area and the estimated leaf area using the linear model selected (leaf area = 0.000199 + 1.277 L×A25).

Table 10. Goodness of fit of the simple regression model 3 (y = a +c x) proposed to estimate the leaf area (y) depending on the independent variable utilized (x). Analysis of regression residuals (normality, homoscedasticity and independence test).

Independent variable	R^2	Standard error	a	c	Independence	Normal distribution	Homoscedastic
L	76.28	0.001498	−0.002464	0.024730	NO	NO	NO
A	52.46	0.002122	−0.001259	0.680298	NO	NO	NO
A25	77.24	0.001468	−0.00244	0.666715	YES	NO	NO
A50	40.14	0.002381	0.000366	0.532653	NO	NO	NO
A75	16.29	0.002815	0.002771	0.443820	NO	NO	NO
L×A	76.12	0.001503	0.001503	1.41210	YES	NO	YES
L×A25	92.53	0.000840	0.000199	1.27743	YES	YES	YES
L×A50	79.59	0.001390	0.000427	1.54129	YES	NO	YES
L×A75	56.10	0.002038	0.001578	1.90872	NO	NO	NO
A×A50	56.58	0.002027	0.002027	38.6951	NO	NO	NO
A×A75	42.71	0.002342	0.002342	51.8716	NO	NO	NO
A25×A75	46.70	0.002246	0.002246	41.3314	NO	NO	NO

Table 11. Goodness of fit of the simple regression model 4 (log y = a +c logx) proposed to estimate the leaf area (y) depending on the independent variable utilized (x). Analysis of regression residuals (normality, homoscedasticity and independence test).

Independent variable	R^2	Standard error	a	c	Independence	Normal distribution	Homoscedastic
L	85.66	0.135	-1.457	1.720	YES	NO	NO
A	60.16	0.225	1.427	1.869	NO	NO	NO
A25	80.55	0.157	1.384	1.919	YES	NO	NO
A50	62.41	0.219	1.168	1.726	NO	NO	NO
A75	32.04	0.294	0.400	1.232	NO	NO	NO
L×A	86.55	0.131	0.333	1.055	YES	NO	NO
L×A25	93.45	0.091	0.161	1.018	YES	NO	NO
L×A50	90.07	0.112	0.332	1.048	YES	NO	NO
L×A75	81.21	0.154	0.603	1.071	YES	NO	NO
A×A50	72.82	0.186	1.978	1.065	NO	NO	NO
A×A75	59.10	0.228	1.972	1.015	NO	NO	NO
A25×A75	66.39	0.207	1.733	0.976	NO	NO	NO

dates as plants got older as the year progressed.
During the irrigation season, it should be highlighted that the growing conditions were not influenced by external factors, such as extreme climatic conditions or water stress, among others. In fact, the farm work and crop operations followed the traditions of the farmers in the area (De Juan *et al*., 2003), and the crop was maintained free of diseases and weeds. The crop growing stages, which were monitored using the phenological scale proposed by Feller *et al*. (1995), showed that plants were growing as expected at each stage. In this experimental study, the onset of bulbing occurred at 75 days after emergence (DAE). Leaves continued to expand thereafter until 110 DAE (bulbing stage), when the maximum LAI was reached. The highest LAI values were close to 1.72

m^2 leaf m^{-2} soil. After the maximum LAI was reached, the following stage lasted around 20 days (until 130 DAE) as the LAI values decreased rapidly in the last stage, when the leaves started to die. At the end, LAI values were close to 0.50 m^{-2} leaf m^{-2} soil. These LAI values were very similar to the values obtained by Tei *et al* (1996), who explained that the cessation of appearance of new leaf blades occurs with the onset of bulbing.

Model 3 was very similar to the model obtained for an onion crop by Gamiely *et al*. (1991), and the authors selected a model "y = a+cx", considering total leaf length (L) as the independent variable. Other authors such as Hoffman (1971), developed a model "log y = a + c logx" also using L as the independent variable.

In all the proposed models, sampling occasion 4 showed the highest differences between predicted and measured leaf area, which can be explained by most of plants being at the leaf senescence stage.

It is expected that the models proposed in this paper can be used in other areas where growing conditions are normal. In situations with restriction of water or fertilizer application, or crops with diseases and weeds, the use of these models is not recommended.

Linear regression models that use leaf length and width are the most commonly used models and parameters for determining leaf area in several crops. Cittadini and Peri (2006) estimated leaf area using leaf length and width for sweet cherry. In this case, linear regression equations were fit using the length, width and their product as independent variables. Olfati *et al*. (2009) obtained leaf area estimates of red cabbage based on predictive equations derived from linear measurements of leaf length and width and their combination. A linear equation with width as the independent variable provided the most accurate estimate of leaf area in this study. In different Pecan cultivars, Torri *et al*. (2009) used regression linear models to determine leaf area, using the length and width as independent variables.

The use of non-linear regression models is less extensive for estimating leaf area. Thus, Antunes *et al*. (2008) used non-destructive measurements of leaf length and width for estimating the area of leaves of eight field-grown coffee cultivars. In this case, they obtained better results using power models than linear models.

Using mathematical models for estimating the leaf area would be an easy and time efficient non-destructive method for users. This approach could be useful for managers in agriculture and can be regarded as a decision support system tool since it can help in monitoring crop growth, while providing information for farmers on crop growth and development, water demand or biomass production. Such a mathematical model for estimating the leaf area reduces sampling effort and cost, and may increase precision where samples of leaf size are difficult to handle.

The use of these models is difficult to introduce in agriculture, due to the low technical knowledge of some producers. In spite of this, most of water users associations are advised by technicians, who would make the introduction of these tools easier. Moreover, several decision support system tools, such as irrigation advisory services, are now working in irrigable areas, so the use of these models might be included as a complementary activity of the irrigation advisory services, helping producers to use them.

Conclusions

The combined application of multivariate techniques such as PCA and CA for grouping leaf parameters was useful in reducing the number of variables from 25 to 12. Among the mathematical models proposed in this paper, the GLM explained a high proportion of high leaf area variability and fulfilled the model assumptions of normal distribution and homoscedasticity. The simple linear regression model "y = a+cx" yielded very similar results to those obtained with the GLM. Thus, both models were useful non-destructive methods for estimating leaf area. Model 3 ("leaf area = 0.000199 + 1.277 L×A25") was the best predictor of the leaf area. The use of mathematical models to estimate the leaf area is a non-destructive, easy and time efficient method for calculating the leaf area. It may be useful for farm managers and farmers, and can be regarded as a decision support system tool since it could be used to monitor crop growth and provide information to farmers on crop growth and development, water demand, weed control, and biomass production, among others.

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