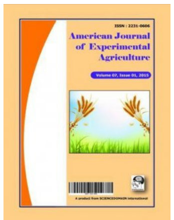

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Estimating the Leaf Area of Cotton (*Gossypium hirsutum* L.) Plants by Means of Relationships between Monopodial and Sympodial Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CJF and HDRC designed the study, wrote the protocol, managed the analyses of the study and the literature searches. Author HDRC performed the statistical analysis and wrote the first draft of the manuscript. Author WJG formatted the manuscript for publication. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Relationships between areas of monopodial and sympodial leaves reported in the literature suggest a consistent size gradient among leaves along the sympodial branches of cotton plants. The objective of this study is to assess how well a non-destructive procedure based on these relationships can estimate leaf area of cotton plants.
Study Design: Randomized complete block with 6 replications.
Place and Duration of Study: The study was conducted in the Drought Tolerance Laboratory at the Texas A&M AgriLife Research and Extension Center near Corpus Christi, TX and at the Texas AgriLife Field Laboratory in Burleson County located 12.9 km west of College Station, TX between February 2014 and July 2015.
Methodology: In 2014, plants from four different cultivars were sampled at the Drought Tolerance Laboratory at the Texas A&M AgriLife Research and Extension Center at Corpus Christi in order gather data to develop the methodology. Four cotton cultivars included PHY 375, PHY499, DPL912,

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and DPL1044. The inclusion of four cultivars had the purpose of incorporating variability in the data to be used for developing the empirical model to estimate WPLA, thus making it more robust. In 2015, field- and lab-grown plants were sampled to evaluate the accuracy of the non-destructive method to estimate whole-plant leaf area.

Results: The results indicated that the methodology overestimated the leaf area of the field-grown plants, while underestimated that of lab-grown plants. However, estimated vs. observed deviations from the 1:1 line were not significant.

Conclusion: It is concluded that despite inconsistencies in leaf area ratios across sympodial branches, the non-destructive method developed still has the potential to be utilized to estimate the leaf area of cotton plants when no leaf area measurement equipment is available.

Keywords: Vegetative framework; main-stem leaves (MSL); fruiting branch leaves (FBL); leaf dimensions.

1. INTRODUCTION

Leaf area is an important parameter for researchers interested in investigating plant performance in any given set of environmental conditions. The reason relies on the fact that plant leaf area production directly affects exchanges of mass and energy between the plant and its environment [1].

The cotton plant (*Gossypium hirsutum* L.) has unique features when it comes to its vegetative framework (Fig. 1). Cotton plant leaves can be divided in two groups: monopodial and sympodial leaves [2]. Monopodial leaves are attached to the main-stem of the plant and frequently called main-stem leaves (MSL), while sympodial leaves are attached to the fruiting branches and commonly referred to as fruiting branch leaves (FBL).

There are several destructive and non-destructive methods to measure or estimate leaf area of individual plants [3]. In general, destructive methods involve detaching leaves from the plant, while in non-destructive procedures leaves are preserved in the plant. Non-destructive methods may involve measurements of leaf dimensions that can be input in empirical equations to estimate leaf area [4]. Several combinations of measurements and models relating leaf length and width to area

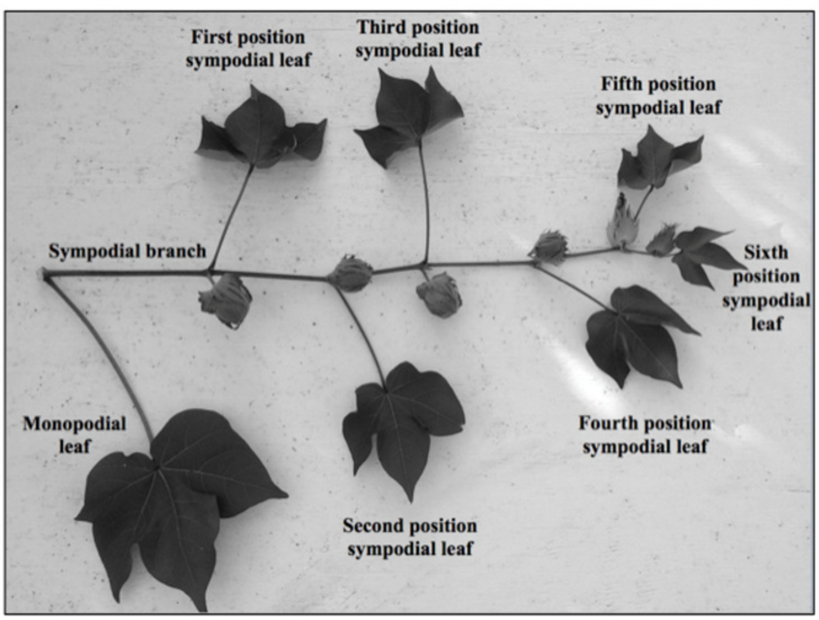

Fig. 1. Schematic view of a sympodial branch of a cotton plant and its monopodial and sympodial leaves

have been developed for different plant species [5,6,7]. Other interesting relationships between MSL and FBL have been found in cotton plants. Constable and Oosterhuis [8] cited earlier studies [9,10,11] that FBL are smaller than the corresponding MSL by a factor of 0.55, 0.4, and 0.3 for the first three positions, respectively. These ratios may have the potential to be incorporated in non-destructive methods for estimating leaf area of individual plants. Furthermore, for cotton growing in field conditions the leaf area index (LAI) can be calculated by extrapolation of plant leaf area measurements or estimates and plant stand [12].

We hypothesize that, if the ratios between MSL and FBL are sufficiently consistent, then they can be applicable for estimating the whole-plant leaf area (WPLA) of cotton plants when combined with non-destructive measurements of MSL areas. The objectives of this study are to assess a) the accuracy of an empirical relationship between length of central vein and blade area in MSL, b) the consistency of the relationship between areas of MSL and corresponding FBL, and c) how a well a procedure based on the relationships between MSL and FBL can estimate WPLA of cotton plants.

2. MATERIALS AND METHODS

The study was conducted at the Drought Tolerance Laboratory near Corpus Christi. Four cotton cultivars, namely PHY 375, PHY499, DPL912, and DPL1044, were planted on February 17th, 2014. The inclusion of four cultivars had the purpose of incorporating variability in the data to be used for developing the empirical model to estimate WPLA, thus making it more robust. Each cultivar had six replicates, totalizing 24 plants.

Plants were grown individually in 13.5-L pots. All pots were filled with 10.8 L of dry fritted clay, which was chosen as the soil medium because of its homogeneity and large volumetric holding capacity, 0.46 L L^{-1} [13]. The soil surface was leveled and covered with finely perforated aluminum foil (60 uniformly distributed needle-size perforations) to allow a uniform distribution of irrigation water across the soil surface. Pots were irrigated in excess before planting. Each pot was planted with four pre-germinated seeds. When plants reached the third true leaf stage, the plant stand was thinned to one per pot. A bamboo stick was inserted at the center of the pot for plant support.

Test plants were irrigated individually with a modified Hoagland's nutrient solution [14]. The nutrient solution contained 224, 62, and 248 ppm of N P K, respectively. From emergence (February 27th) to First Square stage (April 21st), irrigation regime was 1 L day^{-1}. From First Square to First Bloom stage (May 12nd), irrigation regime was changed to 3 L day^{-1}. Other management practices such as pest control were performed as needed.

Leaf area measurements were made once plants reached the First Bloom stage. The potted plants were moved to an air-conditioned laboratory to minimize loss of turgor pressure after leaf blades were detached from the plant, which could affect the measurements. The areas of detached MSL and FBL blades were measured with a LI-3100C Area Meter (LI-COR Inc., Lincoln, NE). Length of the central vein of individual MSL was measured using a ruler.

Three types of functions (linear, 2nd degree polynomial, and power) were used to obtain the regression of MSL area on central vein length. Since area must equal zero at zero length, the intercepts were set to zero in the linear and polynomial functions. The slopes (β) of the lines were subjected to the analysis of variance (ANOVA) in order to test their significance.

The ratio values between MSL and FBL were calculated by dividing the area of each successive FBL by the area of their corresponding MSL. The 95% confidence interval (95% CI) for the means of these ratios were calculated in order to check the agreement of these values with those reported by Constable and Oosterhuis [8].

The accuracy of the method to estimate MSL area WPLA was assessed in 2015 using field-grown and lab-grown plants of cultivar PHY 375. Ten field-grown plants at the cutout growth stage were collected on July 21st, 2015 from rain-fed plots at the Texas AgriLife Field Laboratory in Burleson County located 12.9 km west of College Station, TX. Another six lab-grown plants at similar growth stage were collected on July 27th 2015 from an ongoing water stress study conducted in the Drought Tolerance Laboratory in Corpus Christi, TX. The field-grown plants were planted on April 9th, at a rate of 100,000 plants per hectare with rows spaced at 1.02 m apart. The plants collected at the Drought Tolerance Laboratory were grown individually in 13.5-L pots as described above. Field-grown plants received two applications of 32-0-0 fertilizer (one on March 6th at a rate of 100 lb per acre, and another on May 23rd at a rate of 50 lb per acre) and one application of plant growth regulators cyclanilide (3.3 g a.i. ha^{-1}) + mepiquat chloride (13 g a.i. ha^{-1}) on June 25th, when plants were blooming. In each of 16 plants collected, the length of the central vein in each MSL was measured and the number of FBL in the corresponding sympodium was recorded. The WPLA for each plant was measured with the LI-3100C Area Meter (LI-COR Inc., Lincoln, NE) by scanning each leaf individually. Therefore, the method for estimating WPLA involves a three-step procedure. First, at a given sympodial node the length of the central vein of the MSL is measured and its area is estimated by the regression of MSL area on central vein length. Second, the areas of the FBL in the same sympodial branch are calculated by multiplying the estimated MSL area by the corresponding FBL: MSL area ratio for the leaves present in that sympodium. Third and last, WPLA is calculated as the sum of all MSL and FBL estimated areas.

The data was summarized using Excel 2010 (Microsoft Inc., Redmond, WA) and analyzed using JMP Pro 11 (SAS Institute Inc., Cary, NC).

3. RESULTS AND DISCUSSION

3.1 Estimate of MSL Area from Leaf Dimension

All regression equations relating blade area to length of central vein in MSL are significant at the 1% probability level (Table 1), but the one best explaining the relationship (highest R2 value) was the power equation (Fig. 2).

3.2 Assessing Consistency of Area Ratios between FBL and MSL

The FBL: MSL area ratio for the 1st FBL was similar to the one reported by Constable and Oosterhuis [8]. However, the area ratios for the second and third position FBL were significantly different from the values reported these authors (Table 2). These differences may be attributed to a range of factors, such as cultivar, fruit set distribution, and environmental differences. Nevertheless, and similarly as reported by Constable and Oosterhuis [8], FBL: MSL leaf area ratio values suggest a declining leaf size gradient along the fruiting branch. On average, the leaf area ratios for FBL positions 1 to 4 decrease about 20% from one position to the next, and about 40% between FBL 4 and 5.

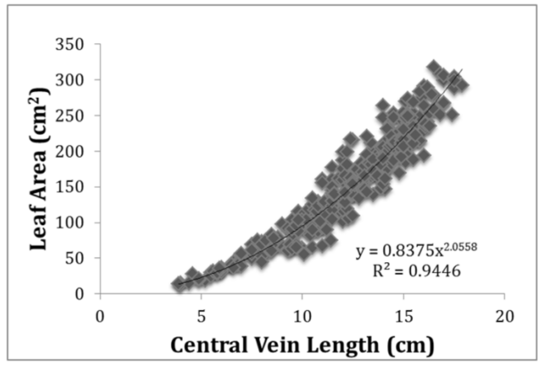

Fig. 2. Regression equation of main stem leaf area on length of its central vein length
Data from four cultivars (n = 337)

3.3 Verifying the Accuracy of the Method for Estimating MSL Area and WPLA

The area of MSL was estimated using the best-fitted regression of blade area on length of the central vein ($A = 0.8375*length^{2.0558}$) as shown in Table 1 and Fig. 2. The areas of each FBL was calculated as $A= (0.8375*length^{2.0558})*$ (FBL: MSL Ratio). FBL: MSL ratios are shown in Table 2.

The power regression equation was effective in estimating the area of the main-stem leaves from length of the central vein at both locations (Fig. 3). Regressions showed high R^2 values; 0.773 for data from field-grown plants (Fig. 3A) and 0.957 for data from lab-grown plants (Fig. 3B), both significant at 1% probability. The regression slopes for the main-stem leaves sampled from field-grown plants and lab-grown plants were 0.9677 and 1.0111, respectively, and not significantly different from the 1:1 line (P=0.4426 and 0.6805, respectively). We have not found published references related to this method for estimating MSL area in cotton using the length of the central vein. This method provides a simpler and yet effective technique than the one employing length of the main vein and maximum width of the leaf blade as reported by Fernandez et al. [7].

On average, WPLA was 12.6% underestimated for field-grown plants sampled in College Station (Table 3). The mean difference between estimated and measured values, however, was not significantly different at the 5% probability level. Conversely, on average WPLA was 10.8% overestimated for lab-grown plants sampled in Corpus Christi (Table 4). The mean difference between estimated and measured values was not significantly different at the 5% level as well.

For both locations, the regressions of estimated on measured showed that departures from the 1:1 line were not significant (Fig. 4). The regression slopes for field-grown and lab-grown plants were 0.8618 and 1.1102, respectively. Both slopes were not significantly different from the slope of the 1:1 line at 5% probability, *P=0.109* and *P=0.09*, respectively.

Since the method for estimating the area of MSL showed good fitness between estimated and observed values, the deviations observed between estimated and observed WPLA values in the two locations can be best explained by the variability in the FBL: MSL area ratios. These ratios vary among sympodial branches as shown by the 95% CI in Tables 5 and 6. The field-grown plants sampled in College Station had higher

Table 1. Significance of the slopes of the regression equations relating leaf blade area to length of leaf central vein**

Model	Regression equation	Regression parameters	
		R2	P-value for β
Power	$y = 0.8375x^{2.0558}$	0.94	<0.01**
Polynomial	$y = 0.9915x^2–0.1941x$	0.91	<0.01**
Linear	$y = 13.193x$	0.74	<0.01**

*** = Significant at the 1% probability level; n = 337 observations*

Table 2. Confidence interval (CI) for the ratio between the fruiting branch leaves (FBL) and their corresponding main-stem leaf (MSL), and comparison between the ratio means and values reported in the literature. Data from four cultivars were combined*

Ratio type	Data from the four cultivars combined						
	Literature values	Mean	Mean difference	P-value	Lower 95% CI	Upper 95% CI	n
1st FBL:MSL	0.55	0.55	0.00ns	0.972	0.52	0.59	198
2nd FBL:MSL	0.4	0.45	0.05**	0.003	0.42	0.49	166
3rd FBL:MSL	0.3	0.36	0.06**	0.002	0.32	0.40	109
4th FBL:MSL	-	0.28			0.23	0.34	48
5th FBL:MSL	-	0.17			0.03	0.31	8

**ns = non-significant at the 5% probability level; ** = significant at the 1% probability level; n = number of observations*

Table 3. Measured and estimated whole-plant leaf area (WPLA) values for the samples from college station, TX (2015)*

Sample	Estimated	Measured	Underestimation
	m^2		%
1	0.213	0.238	10.2
2	0.183	0.206	11.4
3	0.203	0.218	7.0
4	0.156	0.171	8.8
5	0.232	0.255	9.1
6	0.284	0.361	21.3
7	0.165	0.201	18.0
8	0.241	0.270	10.8
9	0.246	0.290	15.2
10	0.269	0.314	14.2
Mean	0.219	0.252	12.6
Mean diff.	0.033ns		
Prob > |t|	0.1623		

**ns = not significant at the 5% probability level*

FBL: MSL area ratios for the second, third, and fourth sympodial leaves than those used in the calculations (Table 5). Consequently, the method underestimated WPLA for these field-grown plants. Conversely, the lab-grown plants sampled in Corpus Christi had lower FBL: MSL area ratios for the first, second, and third sympodial leaves than those used in the calculations (Table 6), thus resulting in overestimation of WLPA for these plants. The regression slopes of estimated on measured WLPA for field-grown and lab-grown plants were 0.8618 and 1.1102, respectively, (Fig. 4). Departures of these regression slopes from the 1:1 line were not significant at 5% probability.

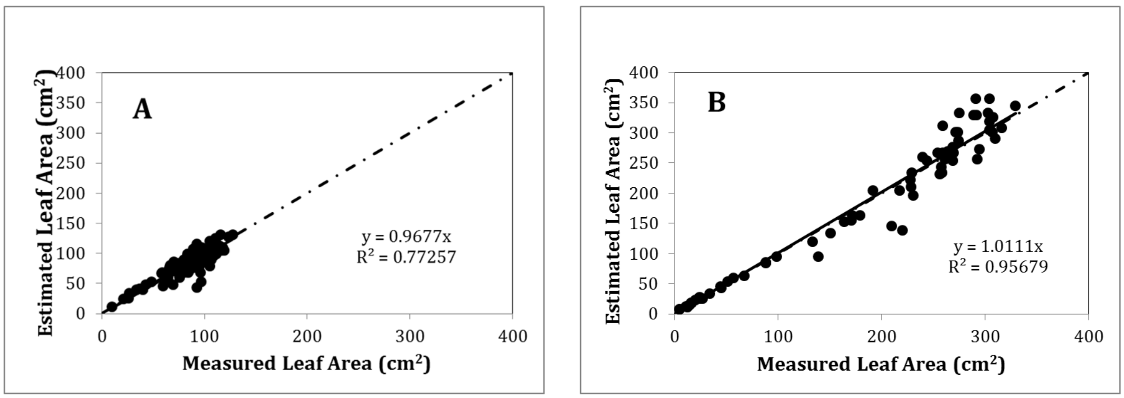

Fig. 3. Regressions of estimated main-stem leaf area on measured main-stem leaf area for (A) field-grown plants sampled in college station and (B) laboratory-grown plants in Corpus Christi

Regression intercepts were set to zero. The dashed line represents the 1:1 line

Table 4. Measured and estimated whole-plant leaf area (WPLA) values for the samples from Corpus Christi, TX (2015)*

Sample	Estimated	Measured	Overestimation
	m^2		%
1	0.345	0.325	6.1
2	0.423	0.381	11.0
3	0.446	0.383	16.4
4	0.398	0.361	10.1
5	0.397	0.361	10.0
6	0.337	0.304	10.9
Mean	0.391	0.353	10.8
Mean diff.	-0.038ns		
Prob > |t|	0.1108		

**ns = not significant at the 5% probability level*

Table 5. Confidence interval (CI) for the ratio between the fruiting branch leaves (FBL) and their corresponding main-stem leaf (MSL), and comparison between the ratio means for plants sampled in college station, 2015*

Ratio type	Data from plants sampled in CS						
	Values used in calculation	Mean	Mean difference	P-value	Lower 95% CI	Upper 95% CI	n
MSL:1st FBL	0.55	0.59	-0.04ns	0.1138	0.56	0.62	106
MSL:2nd FBL	0.45	0.55	-0.10**	0.003	0.50	0.60	75
MSL:3rd FBL	0.36	0.59	-0.23**	<.0001	0.53	0.66	45
MSL:4th FBL	0.28	0.48	-0.20**	<.0001	0.41	0.56	23
MSL:5th FBL	0.17	0.33	-0.16ns	0.1627	0.03	0.64	4

**ns = non-significant at the 5% probability level; * = significant at the 5% probability level; ** = significant at the 1% probability level; n = number of observations*

Table 6. Confidence interval (CI) for the ratio between the fruiting branch leaves (FBL) and their corresponding main-stem leaf (MSL), and comparison between the ratio means for plants sampled in Corpus Christi, 2015*

Ratio type	Data from plants sampled in CC						
	Values used in calculation	Mean	Mean difference	P-value	Lower 95% CI	Upper 95% CI	n
MSL:1st FBL	0.55	0.47	0.08*	0.0413	0.40	0.54	44
MSL:2nd FBL	0.45	0.35	0.10*	0.0104	0.28	0.42	27
MSL:3rd FBL	0.36	0.18	0.18**	0.0041	0.12	0.24	11
MSL:4th FBL	0.28						
MSL:5th FBL	0.17						

**ns = non-significant at the 5% probability level; ** = significant at the 1% probability level; n = number of observations*

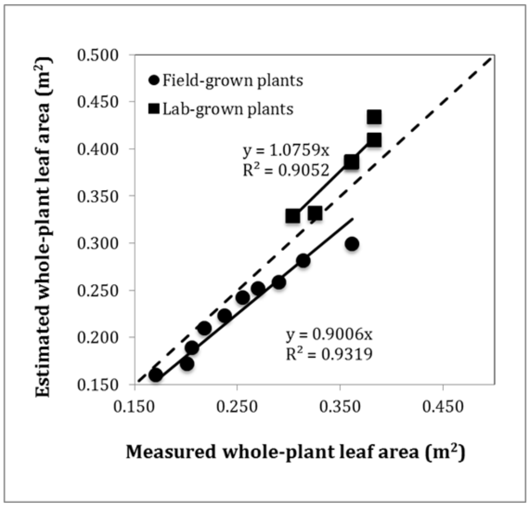

Fig. 4. Regression of cotton whole-plant leaf area (WPLA) estimated by a non-destructive method on WPLA measured destructively for field-grown plants sampled in college station and lab-grown plants sampled in Corpus Christi during 2015

The dashed line represents the 1:1 line. The slopes are not significantly different from 1 at the 5% probability level

4. CONCLUSION

These results showed that the method's assumption that FBL: MSL area ratios are stable across sympodial branches is a simplification that leads to errors in the estimates of WPLA in cotton. These ratios vary among sympodial branches as shown by the 95% CI in Tables 2, 5 and 6. The method was designed to provide a simplistic approach to estimate WPLA when no instrumentation is readily available, and incorporation of additional information to decrease this source of variability would require the collection of much larger data sets, which would defeat the original purpose of the method. Nevertheless, since deviations from the 1:1 line of estimated vs. observed values for both field-grown and lab-grown plants were not significant, it can be concluded that this method of estimating WPLA is capable of yielding reasonable good approximations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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