

LEAF AREA DETERMINATION IN BUSH BEAN

(*Phaseolus vulgaris* L.)¹

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ABSTRACT

Leaf area of bush bean cultivars 'Harvester' and 'Topcrop' were estimated by (i) disc method, (ii) linear measurements, and (iii) dry weight method. One hundred complete discs of diameter 20.3mm devoid of large vascular bundles provided an accurate estimate of leaf area. The presence of midrib and large vascular bundles imparted significant error. Linear measurements of leaves (length and width) could also be used effectively in the estimation of leaf area. Leaflet length $\times$ width product provided the best estimate of leaf area. Leaflet width was also an accurate predictor of area, but the relationship between leaflet area and unconverted length and width measurements of one plant was found unsuitable for leaf area estimation of other plants. Leaf dry weight is also an accurate predictor of leaf area. Leaf area of 'Harvester' and 'Topcrop' can be estimated using a single common linear relationship.

INTRODUCTION

An essential component of plant growth analysis is the determination of 'assimilatory surface area' or broadly 'leaf area'. Over the years various methods have been developed for quick and sufficiently accurate determination of leaf area. Accordingly, leaf area could either be assessed directly or estimated indirectly using mathematical relationships between leaf area and leaf characters.

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Of the estimation methods, 'disc method', 'dry weight method', and 'linear measurements' appear to be the most frequently used, probably because these methods do not require expensive equipment. In the disc method of leaf area determination, the size and number of discs used (Nur, 1971) and the mode of extraction (Watson and Watson, 1953, and Kvet and Marshall, 1971) have been found vital for accurate estimation. The midrib and large vascular bundles of leaves have been found to have introduced large errors in the estimation of leaf area in sugar beet and have been rectified by either excluding the midrib (Kvet and Marshall, 1971) or by counting those discs which were more than half complete as complete ones (Watson and Watson, 1953). However, it has been reported that in potato the bias imparted by inclusion of the midrib was negligible (Bremner and Taha, 1966; Gunasena, 1969). Several forms of regression equations have been described for leaf area estimation using linear measurements and the linear form $Y = a + b \times$ has been the most commonly used one (Kvet and Marshall, 1971) utilizing leaf length, leaf width, or length $\times$ width product as the $\times$ variable.

The present study was undertaken as a preliminary investigation in order to develop a quick, accurate, and inexpensive method to determine leaf area for growth analysis purposes in bush bean.

MATERIALS AND METHODS

The experiment was carried out at the Department of Horticultural Science, University of Saskatchewan, Canada, commencing March, 1980.

Raising of plants

Bush bean cultivars 'Harvester' and 'Topcrop' were raised in the greenhouse in pots of diameter 12.7cm. The growing medium was 1:1:1 mixture by volume of peat moss, sand and soil. Five seeds were sown in each pot and thinned out retaining the two most vigorous seedlings eight days after sowing. Irrigation was done twice a day and plants fertilized once a week using a water soluble 20-20-20 fertilizer mixture along with irrigation water. A sixteen hour artificial lighting (220 watts. m⁻²) was provided throughout the growing period. Plants at early flowering stage were utilized for leaf area determination.

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Linear measurement

Leaves from 15 'Topcrop' plants were used in this study. Length (base to tip) and width (broadest point at the base) were measured in individual lobes for each plant. The area of the respective leaflets were recorded using a 'LI 300 Portable Area Metre'.

Disc method

Cultivars 'Harvester' and 'Topcrop' were subjected to this investigation. Six samples, each containing leaves from four plants, were examined for both cultivars. Leaf areas of samples were measured with the 'LI 3000 Portable Area Metre' and fresh weights of individual samples were noted. Leaves were piled one over the other and circular discs were extracted driving a borer of 20.3mm internal diameter. Firstly 10 groups of 10 complete discs were chosen at random. The groups were weighed separately, the subsequent groups were combined and cumulative weights were also recorded. Secondly, complete discs devoid of large vascular bundles were selected on a visual estimation and grouped into 10 lots of 10 discs each and weights recorded as before. In the succeeding discussion the former category of discs will be termed 'unselected' and the latter will be called 'selected' discs.

The leaf area was calculated using the following formula :

$$\text{Leaf area} = \frac{\text{Cross sectional area of borer} \times \text{Number of discs}}{\text{Total weight of discs}} \times \text{Total weight of leaf}$$

Dry weight method

Leaf area of 30 individual plants of 'Harvester' and 'Topcrop' were measured using the 'LI 3000 Portable Area Metre'. Leaf dry weights were obtained after oven drying the samples at 70°C for 48 hours.

RESULTS AND DISCUSSION

Linear measurement

A summary of the linear dimensions of individual leaflets (length and width) and their area is presented in Table 1. The values of coefficients of variation indicate that there is considerable variation in leaflet length, leaflet width, and particularly leaflet area among leaves within a single plant. This is in accordance with what has been reported by Ackley *et al.* (1958) with bush bean 'Stringless Green Pod' and runner bean 'Blue Lake'.

Regressions were constructed for leaflet area on leaflet length, width, and length $\times$ width product for the central and side lobes separately as well as for the combination of these two types of leaflets (Table 2). Eight out of the nine categories viz. three leaflet types $\times$ three measurement criteria, of regression produced average coefficients of determination over 90% indicating that length, width, and length $\times$ width product of leaflets were effective predictors of leaf area according to linear relationships. Similar phenomena have been observed for several crops (Kvet and Marshall, 1971) including bush bean (Wiendroth and Weage, 1971). Of the different measurement criteria tested it was found that length $\times$ width product produced the most accurate estimate of leaf area as it consistently produced the highest values for coefficients of determination. Accordingly, the second best criterion was leaflet width and the estimation of leaf area using leaflet length ranked third.

Bartlett's test indicated that only two of the nine categories of leaf area estimation produced homogeneous variances of deviation from regression over plants. The χ^2 values were 6.9 and 8.9 for length and width measurements respectively for the centre lobe (with 14 degrees of freedom in both cases). In the other seven instances the χ^2 values exceeded 28.9 and were significant at $P = 0.01$. Consequently, as pointed out by Snedecor and Cochran (1972) it is inappropriate to pool the regression coefficients and the intercepts obtained for different plants to derive a common equation. This suggests that the regressions of each plant should be viewed individually or an appropriate form of transformation should be used in deriving a common equation.

Disc method

The objective of utilizing only the complete discs in this study was for operational convenience and to minimize on human error in the selection of discs.

For 'Harvester' and 'Topcrop' the leaf area estimated using 'unselected' discs was respectively 6.3% and 9.3% lower when compared to the measured leaf area (Table 3). Paired 't' test showed that these differences were statistically significant. However, the leaf areas estimated using 'selected' discs were not significantly different to the measured leaf area with respect to both cultivars and the differences were only 1.4% for 'Harvester' and 0.4% for 'Topcrop' (Table 3). Therefore, it could be

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inferred that the leaf area estimated using complete discs devoid of large vascular bundles was unbiased, whereas leaf area estimation using randomly selected discs (i.e. inclusive of discs containing the midrib and large veins) was erroneous. Watson and Watson (1953) as well as Kvet and Marshall (1971) also observed significant bias imparted by large vascular bundles in the disc method of leaf area determination for sugar beet.

Determination of the number of 'selected' discs required to provide a sufficiently accurate estimate of leaf area necessitated the following steps. Firstly, by establishing the homogeneity of variances of the mean weights of the groups of 10 discs, and it was found that the respective variances were homogeneous when the two cultivars were considered separately and also with the pooled values for the two cultivars (Table 4). Consequently, it was possible to calculate the pooled variance (2.6×10^{-3} g²) and the overall mean weight of discs (0.642g per 10 discs). These values were used to compute the standard errors of 'n' discs as a percentage of overall mean (Figure 1). From this plot it could be extrapolated that to work within a 5.0% confidence interval (i.e. $\pm 2.5\%$) approximately 100 discs would be required.

Dry weight method

The measured leaf area and the leaf dry weight based on 30 individual plants were linearly related with respect to both 'Harvester' and 'Topcrop' (Figure 2). Test of homogeneity for residual variances, intercepts, and the regression coefficients for the two regression lines (ie. two cultivars) were homogeneous, hence allowing the possibility of combining the two equations. Therefore, the leaf area of cvs. 'Harvester' and 'Topcrop' could be estimated using the equation:

$$y = 180.0 + 197.0x$$

where x and y were leaf dry weight (g) and leaf area (cm²) respectively.

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Table 1. Summary of leaflet area and linear measurements from leaves of fifteen plants of bush bean 'Topcrop'.

<i>Parameter</i>	<i>Range</i>	<i>Mean</i>	<i>C. V.¹</i>
Leaflet length (cm)	4.99 — 8.97	6.78	60.1
Leaflet width (cm)	3.41 — 6.64	4.47	73.0
Leaflet area (cm ²)	16.97 — 45.36	27.61	133.7

1 — Coefficient of variation (%).

Table 2. Summary of linear regression analysis between leaflet length (L) leaflet width (W) and leaflet length x width product (L. W.) versus leaflet area of bush bean 'Topcrop'.

<i>Identity of leaflet</i>	<i>Independent variable</i>	<i>Intercept¹</i>	<i>Slope²</i>	<i>C.D³</i>
Centre lobe	L	- 21.34 $\pm$ 1.56	6.47 $\pm$ 0.22	92.6
	W	- 15.01 $\pm$ 1.29	8.71 $\pm$ 0.29	95.4
	L. W	- 0.59 $\pm$ 0.13	0.56 $\pm$ 0.01	99.0
Side lobe	L	- 18.24 $\pm$ 1.39	6.33 $\pm$ 0.17	91.5
	W	- 12.35 $\pm$ 0.88	8.30 $\pm$ 0.27	93.2
	L. W	- 0.64 $\pm$ 0.15	0.65 $\pm$ 0.01	98.0
Composite of all lobes	L	- 20.91 $\pm$ 1.67	7.07 $\pm$ 0.21	83.8
	W	- 14.69 $\pm$ 1.14	9.38 $\pm$ 0.29	93.9
	L. W	- 0.78 $\pm$ 0.14	0.67 $\pm$ 0.01	93.2

1 — Each linear regression analysis based on 14 to 30 points.

2 — Average of 15 estimates.

3 — Coefficient of determination (%).

Table 3. Comparison of measured leaf area with leaf area estimated¹ using 'selected' and 'unselected' discs in bush beans 'Harvester' and 'Topcrop'.

<i>Cultivar</i>	<i>Mean measured leaf area (cm²)</i>	<i>Estimated leaf area (cm²)</i>	
		<i>'Unselected' disc</i>	<i>'Selected' disc</i>
'Harvester'	3123.5	2928.3	3080.0
	t	5.73**	0.35 ^{NS}
'Topcrop'	3099.9	2812.5	3112.4
	t	3.10*	0.14 ^{NS}
Pooled 'Harvester' and 'Topcrop'	t	4.60**	0.35 ^{NS}

1 — Overall average estimated utilizing 10 to 100 discs per sample.

^{NS} — Not significant.

* — Significant at 5.0% level.

** — Significant at 1.0% level.

Table 4. Test of homogeneity of variance¹ (Bartlett's test) on weights of groups of 10 'selected' discs of bush beans 'Harvester' and 'Topcrop'

<i>Item</i>	<i>Cultivar</i>		
	<i>'Harvester'</i>	<i>'Topcrop'</i>	<i>Pooled²</i>
Degrees of freedom	5	5	11
Mean variance	2.4×10^{-3}	2.8×10^{-3}	2.6×10^{-3}
Corrected χ^2	3.59 ^{NS}	9.88 ^{NS}	16.32 ^{NS}

1 — Variances calculated among weights (g) of 10 groups of discs, each group containing 10 discs in each sample. Each cultivar contained six such samples.

2 — Pooled 'Harvester' and 'Topcrop'.

^{NS} — Not significant.

Table χ^2 — 5 d.f (P = 0.05) = 11.07

Table χ^2 — 11 df (P = 0.05) = 19.67

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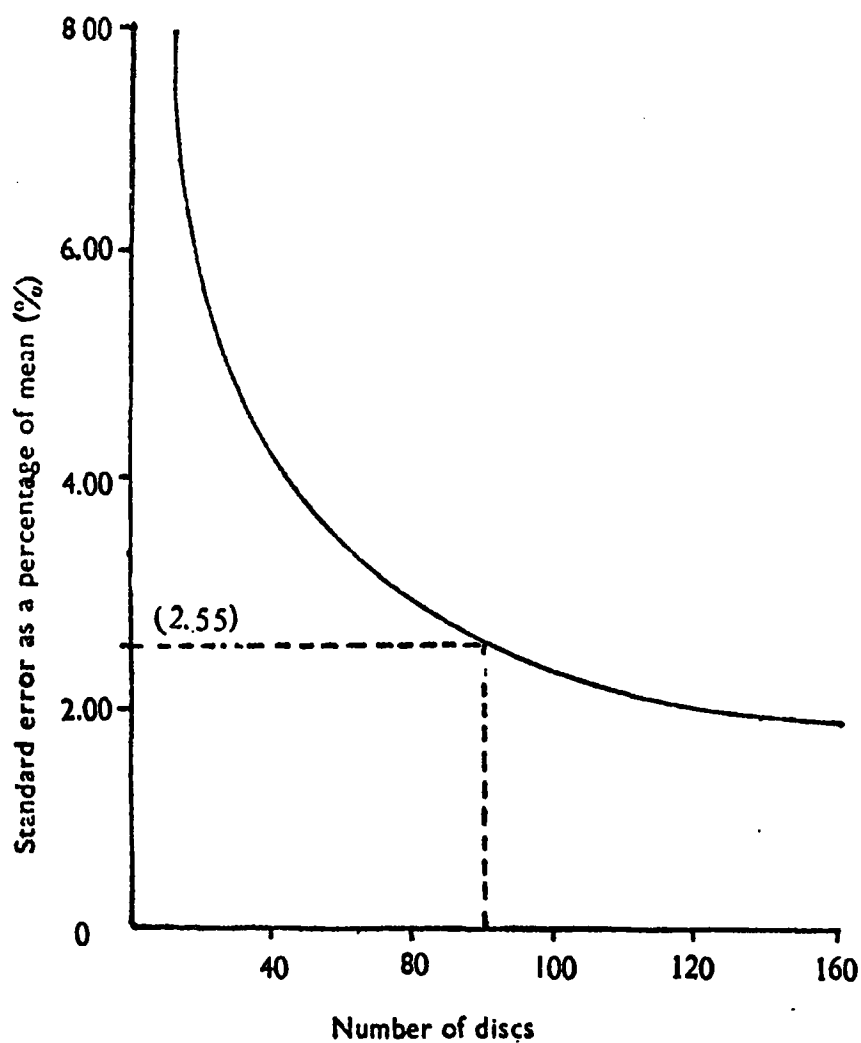


Figure 1. Relationship of standard error expressed as a percentage of mean to the number of selected discs in bush beans ('Harvester' and 'Topcrop').

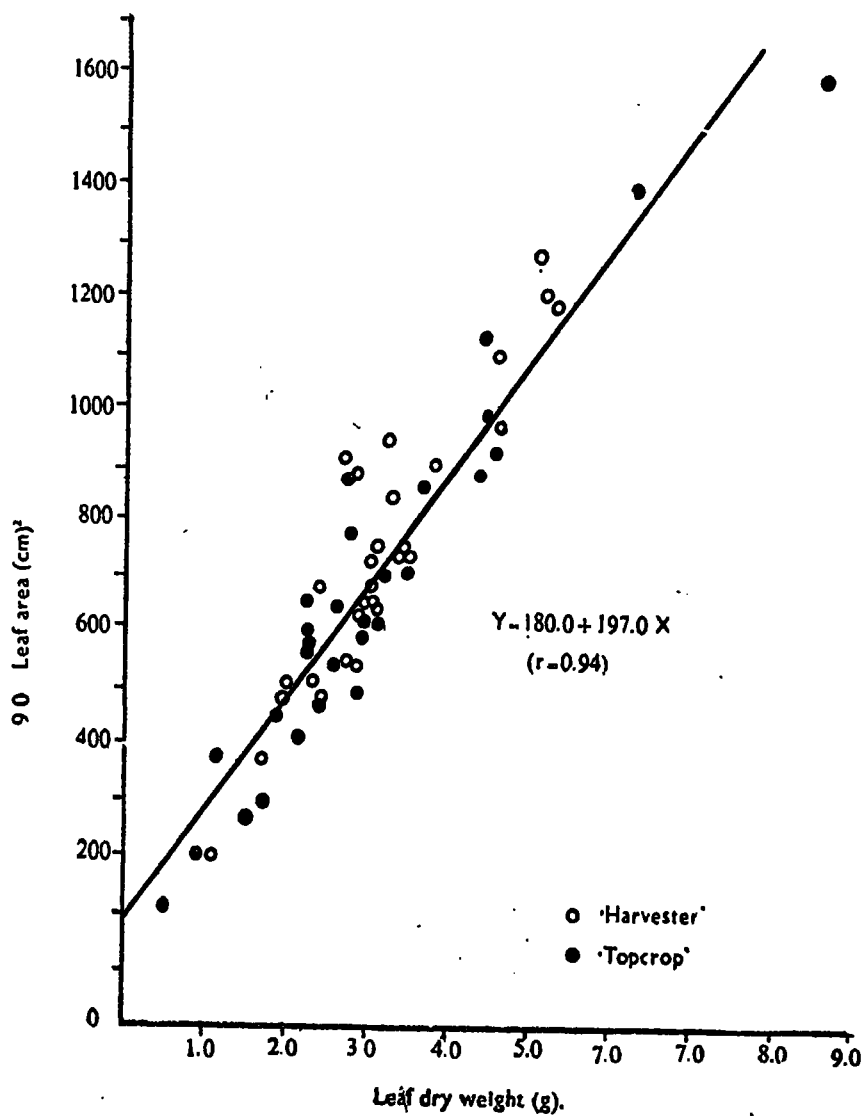


Figure 2. Relationship between leaf dry weight and leaf area in bush beans cultivars 'Harvester' and 'Topcrop'.