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SOME METHODS FOR DETERMINING LEAF AREAS IN *HEVEA*

BY

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SUMMARY

The 'punched disc' method described here gave accurate estimates of leaf areas of all cultivars of Hevea *that were tested.*

Leaf length, maximum leaf width and the product of these dimensions were very highly correlated to leaf area. Highly significant correlations were also observed between area of the central leaflet or its length and the area of the entire trifoliate leaf. The two lateral leaflets of the trifoliate leaf were almost equal in area.

The accuracy and use of these parameters for measuring leaf areas are examined.

INTRODUCTION

The estimation of leaf areas is important in the analysis of plant growth. Accuracy and rapidity are the main criteria determining the choice of any particular method of estimation. Depending upon the nature of the investigation, emphasis may need to be given to one of these factors at the relative expense of the other. For example, in laboratory measurements of photosynthetic rates of leaves accurate measurement of leaf area is more important whilst in the measurements of leaf areas on a large number of plants, rapidity of measurement may supersede accuracy in relative importance. Certain methods available for estimating leaf areas are cumbersome and time-consuming ; therefore unsuitable when large samples have to be handled. Some are also destructive, and not suitable where removal of leaves is not permissible.

Planimetry is considered the most accurate method and is often used as a standard for assessing the accuracy of other more rapid ones. However, planimetry is a slow and tedious process and is also not suitable for attached leaves. Although methods of obtaining impressions of attached leaves such as blue-printing on photo sensitized paper that can facilitate planimetering are reported (Goodall, 1947 ; Ruck & Bolas, 1956) they are cumbersome and expensive. Impressions or outline tracings of leaves could also be cut out and weighed accurately and the leaf area thus estimated.

Many automatic planimeters based on light interception (Gerdel & Salter, 1928 ; Frear, 1935 ; Withrow, 1935 ; Mitchell, 1936 ; Kramer, 1937 ; Milthorpe, 1942 and Maggs, 1956) or air-flow (Jenkins, 1959) have been designed. These methods have, however, several drawbacks as pointed out by Orchard (1961) who also constructed an apparatus devoid of many of the defects in the previous systems.

In the 'punched disc' method (Watson & Watson, 1953 ; Pethiyagoda & Rajendram, 1965) discs are removed from a pre-determined position on the leaf ; the weight/area ratio of the disc from this position is equal to the weight/area ratio of the entire leaf.

Gregory (1921) approximated the shape of the leaves of *Cucumis sativus* to geometric figures, an ellipse for the cotyledons and an irregular hexagon or octagon for the leaves, for calculating the areas. Vyvyan & Evans (1932) measured attached leaves of apples using an 'integrator', which is essentially a transparent grid divided into centimeter squares. Darrow (1932) and Thirumalachary (1940) measured areas of leaves in the field by 'the matching method' which involved the matching of leaves against artificially prepared standards of known area. Freeman & Bolas (1955) found that the apple leaf approximates to an ellipse and used this relationship to design a grid by means of which areas of leaves could be read off directly. Pethiyagoda & Rajendram (1965) showed that, for tea leaves, the product of the rectangular area and a proportionality constant gives a good estimate of leaf area. This relationship was used to construct a grid with which leaf areas could be estimated directly.

Leaf area is correlated to its linear dimensions or the product of these dimensions in several dicotyledons (Goodall, 1947 ; Boynton & Harris, 1950 and Kuzicev 1969) and in grasses (Lal & Subha Rao, 1950, 1951 ; Langer, 1955).

Rapid, simple and reasonably accurate methods of estimation of leaf areas in *Hevea brasiliensis*, especially those that can be used in the field on attached leaves, are useful for the analyses of plant growth. Some methods of estimating leaf areas for *Hevea* are examined in this paper.

MATERIALS AND METHODS

Hevea brasiliensis Muell. Arg. leaves are trifoliate. Leaves for area assessments were obtained mostly from the clonal trials of the Rubber Research Institute of Sri Lanka at Dartonfield. The Tjir 1 clonal seedlings used were from one year old rootstock seedlings from a nursery at Dartonfield. Fully expanded leaves were selected at random from a number of trees of each cultivar.

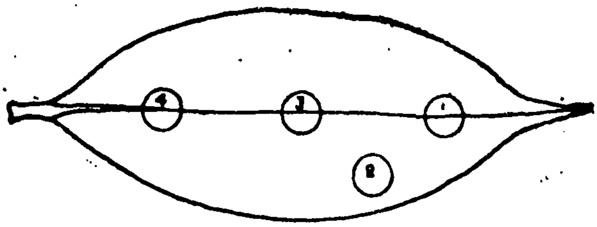
The punched disc method described here is based on the identification of a position on the lamina where the area to weight ratio is the same as that for entire leaves. By determining the weight of a sample of known area, the area corresponding to a known weight of leaves can be determined.

RESULTS

Two tests were made to find the appropriate position of the disc on the leaflet; first, discs 15 mm in diameter were punched from four positions on 10 leaflets of Tjir 1 clonal seedlings as indicated in Fig. 1a. Whole leaflets were weighed before and after the removal of each disc. The calculated mean area of leaflets on the basis of weight/area ratio of each disc, is compared with the area estimated by the planimeter in Table 1. The results indicate that the disc punched at a point $\frac{1}{4}$ of the distance from the apex gave the best estimate of the area of the leaflet, but still exceeded the planimeter values by 6%. In the second test, the disc positions 2 and 4 of the first test were changed as they gave a gross overestimate and underestimate, respectively, of the actual leaflet area. Instead, discs were punched on the midrib at points 1/8, 1/4, 1/3 and 1/2 the distance from the apex (Fig. 1b). These positions were selected because the results of the first test indicated that the disc giving an accurate estimate of the area of the leaflet is likely to be positioned between the midpoint and the tip of the midrib and possibly on the midrib. The results (means for 25 leaflets, Table 1), show that, of the positions tested, the one on the midrib 1/4 the distance from the apex again gave the best estimate of the area. Increased sample size in the second test has decreased the error considerably and overestimates the planimeter value by only 2·5%.

DIAGRAM SHOWING THE POSITIONS FROM WHICH DISCS WERE PUNCHED

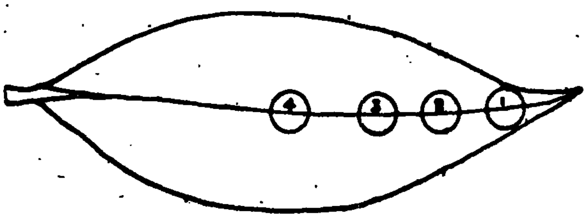
FIG. 1 a (TEST I)



Discs punched —

(1) at 1/4 distance from the apex and on the midrib.
(2) on the lamina.
(3) at the midpoint of the midrib.
(4) at 1/4 distance from the base and on the midrib.

FIG. 1 b (TEST II)



Discs punched at —

(1) 1/8 leaf length from apex
(2) 1/4 leaf length from apex
(3) 1/3 leaf length from apex
(4) 1/2 leaf length from apex

TABLE 1

COMPARISON OF AREAS OF LEAFLETS ESTIMATED BY THE DISC METHOD WITH AREAS MEASURED BY PLANIMETRY

Planimeter Method		Disc Method			
		Position of disc			
Test I		(1)	(2)	(3)	(4)
Mean area (cm^2)	87·7	93·3	109·9	78·7	65·1
%	100·0	106·4	125·3	89·7	74·2
Test II					
Mean area (cm^2)	79·9	89·6	81·9	74·2	67·8
%	100·0	112·1	102·5	92·9	84·9

(See Figs. 1a and 1b for positions of discs)

Using discs punched at 1/4 the length from the apex, areas of 30 leaflets from each of seven clones were determined and compared with areas of the same leaves obtained by planimetry. The data (Table 2) show that this method satisfactorily estimated leaflet areas of the clones tested. The error of estimate is acceptable and for many of the clones is usually a small overestimate. In the case of clone RRIM 701, it is an underestimate of 5%. The distribution of the error of estimates given in Table 3 confirms that this method is reasonably accurate.

TABLE 2

COMPARISON OF LEAFLET AREAS ESTIMATED BY PLANIMETRY AND BY THE DISC METHOD

Mean leaf area (cm^2)

Cultivar	Planimeter Method (a)	Disc Method (b)	b/a x 100
RRIC 45	57·2	59·1	103·3
RRIC 52	66·8	67·1	100·5
RRIC 88	48·6	48·6	100·0
RRIC 36	61·9	62·5	100·7
NAB 15	50·1	50·3	100·4
RRIM 701	55·9	53·4	95·5
PB 86	59·5	62·5	105·0
Mean	57·1	57·6	100·9

TABLE 3

THE DISTRIBUTION OF ERROR FOR THE PUNCHED DISC METHOD

Percent leaflets

Cultivar	% error			
	0 - 5	5 - 10	10 - 15	> 15
RRIC 45	50	23	20	7
RRIC 52	53	29	11	7
RRIC 88	50	37	13	0
RRIC 36	63	30	3	4
NAB 15	83	14	3	0
RRIM 701	64	33	3	0
PB 86	45	37	18	0
Mean	58	29	10	3

In Table 4, the leaflet areas have been categorised into three area groups namely 0 - 40, 40 - 80 and 80 - 120 cm^2 and the corresponding error for each group has been calculated. Except in the case of RRIM 701 the error decreased with increase area of leaflets.

TABLE 4

DISTRIBUTION OF ERROR FOR THE PUNCHED DISC METHOD FOR THREE RANGES OF LEAFLET AREAS

Percentage error

Cultivar	Leaflet area (cm²)		
	0 - 40	40 - 80	80 - 120
RRIC 45	11·0	7·3	2·3
RRIC 52	8·5	5·8	4·8
RRIC 36	9·1	3·9	3·7
RRIC 88	7·1	3·9	3·9
NAB 15	3·7	2·5	2·9
RRIM 701	2·9	5·8	6·1
PB 86	8·1	7·6	2·7
Mean	7·2	5·2	3·8

Leaf area and leaf dimensions

The relationship between the planimetered area and the product of length × maximum width, the length alone and the width alone was tested in ten cultivars. The leaf area was very significantly correlated to each of the parameters in every cultivar used. The results for three cultivars are given in Table 5. The percentage deviations of the estimated areas from the planimetered areas were grouped into five ranges of percent error (Table 6) as a further test for the accuracy of the methods. The results show that the product length into maximum width is a better estimate of leaf area than either length or width ; the length and the width estimates were about the same degree of accuracy.

TABLE 5

RELATIONSHIP BETWEEN PLANIMETERED AREA OF LEAFLET AND ITS LENGTH (L), WIDTH (B) AND LENGTH X WIDTH (L X B)

Cultivar	Parameter	Correlation Coefficient	Regression equation
	l x b	0·997	y = 0·58 + 0·638×
Tjir 1 95*	l	0·944	y = —47·66 + 6·791×
	b	0·962	y = —51·80 + 20·986×
PB 86 60*	l x b	0·995	y = 0·77 + 0·612×
	l	0·979	y = —45·45 + 7·012×
	b	0·974	y = 60·17 × 19·960×
RRIM 701 30*	l x b	0·995	y = 1·30 + 0·687×
	l	0·989	y = 39·99 + 7·493×
	b	0·963	y = 79·29 + 22·087×

y = True area
× = l, b or l x b
* number of leaflets used

TABLE 6

PERCENT PROBABILITY THAT AN ESTIMATE OF THE AREA OF A LEAFLET BY LENGTH, WIDTH OR LENGTH X WIDTH WILL FALL WITHIN SELECTED RANGES OF PERCENTAGE ERROR

Cultivar	Parameter	Percent error				
		0 - 1	1 - 5	5 - 10	10 - 15	>15
Tjir 1 95*	l x b	20·0	55·8	17·9	5·3	1·1
	l	4·2	14·7	29·5	16·8	34·7
	b	7·4	17·9	22·1	24·2	28·4
PB 86 60*	l x b	18·3	60·0	13·3	8·3	—
	l	1·7	33·3	20·0	26·7	18·4
	b	3·3	21·7	33·3	16·7	25·0
RRIM 701 30*	l x b	26·7	50·0	13·3	3·3	6·3
	l	13·3	30·0	33·3	13·3	10·0
	b	3·3	33·3	26·7	13·3	23·3

* Number of leaflets used

Area of lateral leaflets

The central leaflet of the trifoliate leaf is somewhat larger than the lateral leaflet in most cultivars of *Hevea*, and the two laterals appear more or less equal in area. Measurements were made to test whether the lateral leaflets were actually equal in area.

In the first instance, the areas of 15 pairs of leaflets of Tjir 1 seedlings were measured by planimetry. The lateral leaflets were designated left (a) and right (b) according to whether they were on the left or right hand side, respectively, when the leaf is held with the petiole away from oneself.

The total area of 15 leaflets of ' a ' and of ' b ' was 162·9 and 164·9 respectively. The paired t-test showed no significant difference in area between the means of laterals. The validity of this hypothesis was tested on ten cultivars using 50 leaves for each cutivar. Leaflet areas were measured using the length × maximum width method. The results were very similar for all cultivars, and for three cultivars are given in Table 7. The results confirmed that the areas of the two lateral leaflets are nearly equal.

TABLE 7

COMPARISON OF AREAS OF LATERAL LEAFLETS USING THE LENGTH X WIDTH METHOD

Cultivar	Correlation Coefficient	Mean leaflet area (l x b) (cm^2)		$\frac{a}{b}$ x 100
		Left leaflet (a)	Right leaflet (b)	
RRIM 701	0·9280	101·2	99·3	101·90
GT 1	0·9732	51·1	51·2	99·90
RRIC 45	0·9578	81·9	81·4	100·50

Central leaflet and trifoliate leaf

Using the length × width method, areas of 50 trifoliate leaves were measured in each of 9 clones. The area of the central leaflet was very highly correlated to the area of the entire trifoliate leaf for all the clones and the results for three clones are given in Table 8.

TABLE 8

RELATIONSHIP IN AREA (L X B) BETWEEN TRIFOLIATE LEAF AND ITS MID LEAFLET

Cultivar	Correlation Coefficient	Regression equation
RRIC 52	0·9971	y = —18·763 + 2·880×
RRIC 88	0·9895	y = — 9·778 + 2·861×
NAB 15	0·9944	y = — 8·495 + 2·765×

y = area of trifoliate leaf
x = area of mid leaflet

Measuring leaf areas by planimetry on ten trifoliate leaves per clone, it was observed that even the length of the central leaflet was very significantly correlated to the area of the trifoliate leaf (Table 9). The error for all the clones taken together is less than 10% for 73% of the trials (Table 10).

TABLE 9

RELATIONSHIP BETWEEN LENGTH OF THE CENTRAL LEAFLET AND AREA OF THE TRIFOLIATE LEAF

Cultivar	Correlation Coefficient	Regression equation
GT 1	0·9939	y = —137·69 + 18·257×
RRIC 45	0·9904	y = —142·29 + 20·432×
NAB 15	0·9923	y = —149·203 + 24·194×

y = Planimetered area of trifoliate leaf
x = Length of mid leaflet

TABLE 10

PROBABILITY THAT AN ESTIMATE OF LEAF AREA USING LENGTH OF MID LEAFLET AS THE PARAMETER WILL FALL WITHIN THE SELECTED RANGES OF PERCENTAGE ERROR

Percentage error	Percentage probability
0 — 1	11
1 — 5	34
5 — 10	28
10 — 15	10
15 — 25	12
25 — 50	4
<50	1

DISCUSSION

The punched disc method of estimation of area of detached leaves can be used satisfactorily with *Hevea* leaves. Accurate and rapid weighing and punching of discs is necessary in this method to overcome errors caused by drying. The maximum error observed was only about 5%, and usually was in the direction of an overestimate. The data in Table 3 suggest that, theoretically, one possible way of improving the accuracy of the estimates is by the use of discs proportional to the area of the leaves. However, this is not practicable. Perhaps the error could be reduced if the leaves are first sorted out into a few area groups using standard templates. A disc of area optimal for each group can then be used to estimate leaf areas more accurately. The punched disc method is especially suited when the sample size is very large and area of individual leaves is not required.

The regressions of leaf area on linear dimensions (length and maximum width) and the product of these dimensions were highly significant. (Gunasekera, 1971 ; Lim & Narayanan, 1972). Thus, it should be possible to use any one of these criteria for estimation of leaf areas. However, (see Table 6), length × width gives a more accurate estimation of area than either length or width and should be the preferred criterion. Because of the greater error associated with both the length and the width method, their use may be limited to instances where only comparative estimates are required.

In the regression equation relating leaf area to the product of its length and width, the intercepts are of negligible value and may be omitted. The area can then be estimated by multiplying the rectangular area by the regression coefficient. This obviously is not possible with the length or the width method because of the large intercept value. Further, the regression coefficient for each parameter varies from clone to clone, and perhaps within a clone under different environments and treatments. Accordingly it should be necessary to calculate this factor under any particular conditions for each clone when absolute leaf areas are estimated.

The highly significant correlation between area of the entire trifoliate leaf and length of mid leaflet has further simplified measurement of leaf areas. Although not as accurate as the length × width (of each leaflet) method, this method should be especially useful when leaf numbers to be measured are large and absolute accuracy is not required. Leaf area measurements based on linear dimensions are invaluable particularly when detachment of leaves from plants is not permissible.

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