THE DETERMINATION OF LEAF AREAS IN TEA

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The accurate determination of leaf areas is of importance in many types of studies. Leaves have two primary physiological functions—photosynthesis or the building up of complex organic compounds with the aid of light, and transpiration or the loss of water vapour through surface openings or stomata. The intensity with which these two activities progress is related to the total effective area. The many fields of study whose interpretation could involve leaf area measurements include photosynthesis and the role of reserves, growth analysis and shade effects, analytical studies of foliar nutrients, the effect of attack by defoliating insect or fungal pests, water loss and drought resistance and possibly clonal characterization and identification.

There are several methods by which leaf area assessments have been carried out in tea. These are briefly outlined below.

(*a*) Blue-print method

Detached leaves are carefully arranged flat on the sensitive side of a sheet of blue print paper. This is next mounted in a frame with a glass front which presses the leaves firmly against the paper. After exposing the frame to sunlight the paper is suitably developed to yield a permament record of the imprints of leaves. Any type of light-sensitive paper may be used and Figure 1 is an example of a print obtained on photographic paper. Blue-print paper is often selected for reasons of economy and convenience.

Two methods may be used for estimating the areas of the leaf imprints.

(i) *Planimeter method*

The planimeter is an instrument for measuring the area of any plane figure by moving a tracer along the entire length of its boundary line. Planimetry may, therefore, be employed on the blue print impressions.

(ii) *Weighing method*

In this alternative method, the leaf imprints on the paper are carefully cut out and weighed accurately. A standard area of the same paper (say 100 sq cm) is also cut out and weighed. Assuming that the paper is of uniform thickness, knowledge of the weight per unit area enables the area of a piece of known weight to be calculated.

While blue-print methods have the advantage of providing a permanent record of the leaf areas, the actual assessments can be quite tedious. This is especially so when one is dealing with a leaf like that of tea, in which the minute serrations along the margin make both planimetry and cutting out of the leaf imprint accurately, extremely difficult operations. In leaves with simple outlines, this method could be quite satisfactory.

(*b*) The "punched disc" method

As in the blue-print weighing method, the principle here is to determine the weight of a known area of leaf and by a knowledge of the total weight of the leaf sample, to calculate the total area.

A leaf, however, is not of uniform thickness and density over its entire area, allowance having to be made particularly for the midrib which is of proportionately greater weight than the leaf blade. That region of the leaf whose weight per unit area is most closely representative of the total surface has to be determined by trial and error.

To quote an example, the areas of 25 leaves of clone TRI 2016 were estimated individually by punching out discs with a 15 mm diameter cork-borer from four different positions on the leaf. These are—

1 Leaf blade—avoiding midrib,

2 At ¼th distance from the base and including midrib,

3 Half-way along the leaf—including midrib, and

4 At ¼th distance from the apex and including midrib.

The mean area and the corresponding standard error for each estimation is presented in Table 1, alongside a simultaneous assessment by the blue-print weighing method.

TABLE 1—*Comparison between the use of different loci in the punched disc method for determining leaf area.*

	Excluding midrib Lamina	Including Midrib near base	at centre	near apex	Blueprint method
Mean area/leaf in sq cm	45.75	25.04	30.48	38.61	39.57
Standard error	±1.046	±1.082	±0.801	±0.763	±0.825

It is apparent that punching along the midrib at a point one quarter length behind the apex gives the value agreeing most closely with the assessment obtained by the blue-print method. Clearly, the lamina alone gives a gross over-estimate of area while the increasing thickness of the midrib closer to the base, yields an underestimate.

Based upon this and a number of similar observations, in using the punched disc method, a precise adherence to this standard locus has been adopted.

The punched disc method is reasonably accurate and convenient in use. Its disadvantage is that it is necessarily a destructive method as are also blue print methods.

(*c*) **The use of "leaf grids"**

A leaf grid is a device upon which the area of a leaf may be read off directly when it is positioned in a particular manner. The grid has to be specially designed by taking into account certain geometrical characteristics of typical leaves of the particular species. Such a grid has been successfully devised for apple leaves (Freeman and Bolas 1955), and is said to be applicable to all leaves which are approximately elliptical in shape. Tea leaves by and large, belong to this type and the same grid has been used.

In a simpler modification of the method, a series of standards are prepared by cutting outline tracings of a range of leaf sizes on cardboard and determining their areas accurately by planimeter. The area of a leaf is then obtained by visual matching against the standard corresponding with it most closely (Thirumalachary 1940).

These methods have the advantage of being rapid and they do not necessitate the detachment of leaves for measurement. Ideally, a grid should be constructed to suit a particular species. The present paper describes a grid for leaves of tea.

Table 2 gives an indication of the relative accuracies of the four methods described. Fifty leaves were collected at random from each of four clones. The area of each leaf was then assessed by each of the four methods. The procedure was as follows:

(*a*) Its area was read off the grid directly. The grid employed was that devised for apple leaves;

(*b*) a blue-print was made;

(*c*) it was weighed and a punch removed and also weighed;

(*d*) the blue-print was next assessed by planimeter and then cut out and weighed.

Table 2 presents the mean area per leaf as estimated by each of the four methods along with the standard error for the sample.

TABLE 2—*Comparison between four different methods of estimating leaf area. The same sample of fifty leaves from each of four clones was used for each method. The values represent the mean area per leaf in sq cm*

Clone	Planimeter	SE	Punched disc	SE	Blue-print weighing	SE	Leaf area grid	SE
TRI 2024	26.70	±0.91	25.97	±0.86	29.05	±0.96	32.71	±1.06
TRI 2025	41.29	±1.44	37.20	±1.30	45.06	±1.61	48.76	±1.84
TRI 128	13.57	±0.336	13.38	±0.315	15.15	±0.393	18.69	±0.462
TRI 603	26.36	±0.793	25.54	±1.01	28.18	±1.04	32.04	±1.35
Mean	26.98		25.52		29.36		33.05	

(SE = standard error)

Assuming that the planimeter method, although tedious to use, gives the best estimate of area, it is clear that the punched disc method is the best of the other three methods and gives a sufficiently accurate measure for most purposes. Because of its convenience, this latter method has been used as the standard, in efforts to evolve a geometric method for leaf area determinations.

(*d*) **Geometric method**

As has been pointed out above, with the possible exception of the grid method, the other three methods necessitate the removal of the leaf from the plant. In several types of studies, it would be an advantage to be able to estimate the leaf area of a plant without such destructive sampling. To this end it became necessary to work out a formula for determining the area of a leaf, making use of easily measured linear dimensions.

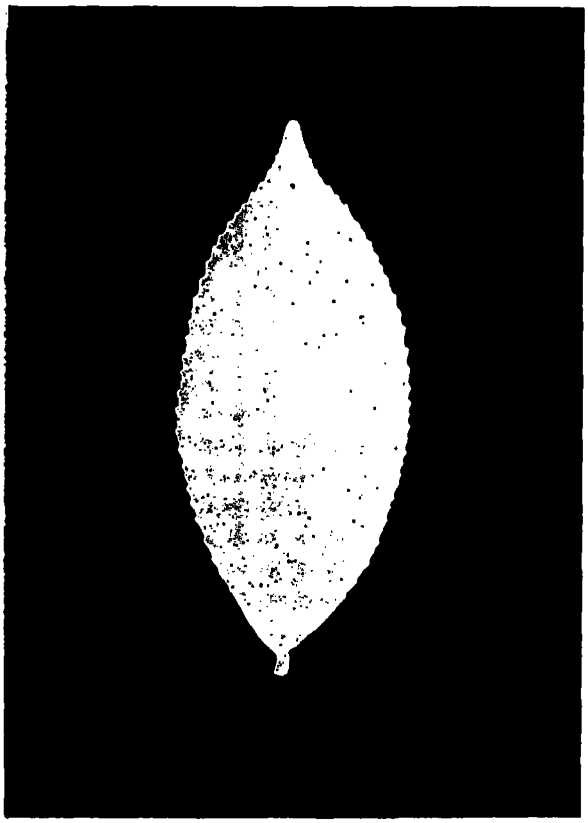

FIGURE 1—*An example of a leaf imprint obtained on photographic paper and suitable for determination of area by planimetry or the weighing method*

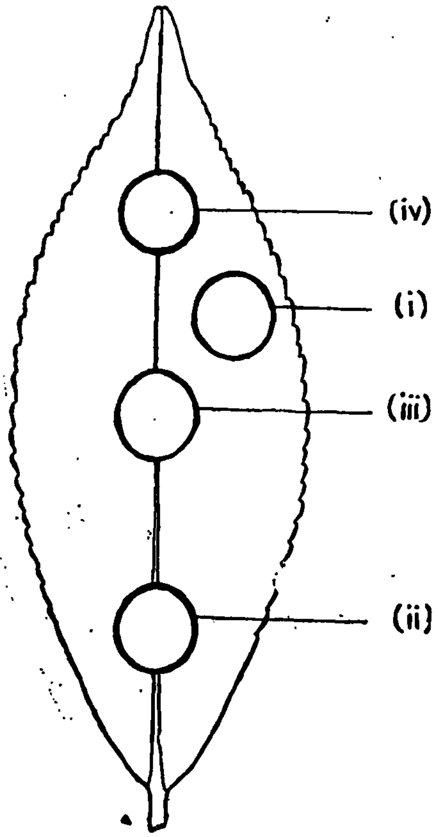

FIGURE 2—*Diagrammatic representation of the different loci that have been tested in area determinations by the "Punched disc" method*

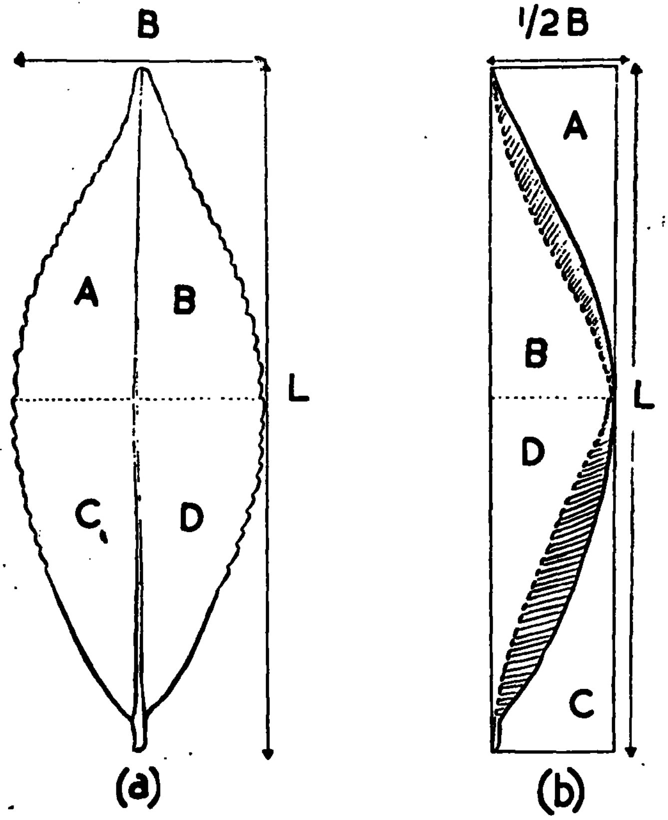

FIGURE 3—*Diagrammatic representation of the manner in which the quadrants of a tea leaf may be re-arranged to form a rectangle—The shaded areas in (b) represent regions of overlap of the quadrants*

The shape of a tea leaf approximates to an ellipse. The leaf area grid that was used had been constructed on the assumption that the shape was a perfect ellipse and therefore the area characterized by the expression, Area = π (a x b), where a and b are the long and short radii.

The large deviations of the estimations by grid, from values obtained by the more reliable methods, indicates that the assumption of an elliptical shape for tea leaves is only approximately valid.

Taking into account the shapes of representative tea leaves, another attempt was made. For this purpose, we assume that the leaf may be divided into quadrants by the midrib and the axis of greatest breadth (Figure 3a).

The quadrants could now be re-arranged in the manner indicated in Figure 3b to form a rectangle whose area would be—

$\frac{1}{2}$ (L x B), where L is the length of the leaf and B is the maximum breadth.

It is clear from the diagram that there is a small area of overlap in this arrangement, which is not allowed for in the above expression. The area so calculated would, therefore, be a slight under-estimate. In other words, the factor used to multiply (B x L) will not be 0.5 but a value somewhat higher.

In order to determine this factor, a small number of mature leaves was collected and their individual areas determined as precisely as possible using the punched disc method and their length and breadth measured.

The areas divided by the product of length and breadth gave a mean value of 0.625 for the factor.

It would be noted that if the tea leaf was a perfect ellipse in shape, its area would be defined by the expression—

$$\pi \frac{L}{2} \times \frac{B}{2} = 0.785 (L \times B)$$

This explains why the grid method (which is designed assuming an elliptical shape) gives an over-estimate of the leaf area for tea (see Table 2). The smaller factor of 0.625 allows for the attenuated tip and base of the tea leaf.

In order to check the general validity of this method, ten tipping shoots were selected from each of ten clones specially chosen to cover the range of leaf shapes commonly encountered in tea. From each shoot, five mature leaves (numbers 4-8) were selected for estimation. Each clone therefore furnished a sample of fifty leaves. Length and maximum breadth were measured for each leaf. From the mean values for length and breadth so obtained, a mean leaf area was calculated, using the expression,

Area = 0.625 (length x breadth).

At the same time mean leaf area for the sample was determined by the punched disc method. The values obtained are presented in Table 3.

TABLE 3—*Comparative leaf areas as estimated by the punched disc and geometric methods—Each figure represents the mean area in sq cm for a sample of 50 leaves comprising leaf numbers 4-8 from 10 shoots for each clone*

	Clone	No of leaves	(*a*) Punched disc method	(*b*) Geometric method	$\frac{(b)}{(a)} \times 100$
1	CH 33	50	33.82	33.70	99.65
2	DUN	50	40.64	39.92	98.23
3	CH 38	50	27.58	28.41	103.01
4	SJ 2/28	50	33.23	33.61	101.14
5	AL 3/4	50	34.46	35.03	101.65
6	SJ 2/30	50	24.98	24.85	99.48
7	UH 34	50	19.60	19.42	99.08
8	EEUD 20	50	31.73	31.77	100.13
9	TRI 2025	50	55.26	54.78	99.13
10	R/GILL	50	24.69	25.63	103.81
	Total		325.99	327.12	—
	Mean		32.60	32.71	100.53

It is clear from the right-hand column that there is very close agreement between the estimates from the two methods. By using the factor 0.625 in the formula, a divergence of not more than 2 or 3% results between the geometric and punched disc methods, for a range of mature leaf shapes.

When the method was applied, however, to samples including leaves which had not all attained their mature shape and size, larger divergences begin to appear when punched disc, geometric and blue-print methods are compared.

Each of the methods described is probably subject to errors when employed on very small and immature leaves. The closeness of agreement between different methods has therefore to serve as an index of reliability. Many critical comparisons of samples comprising the first and second leaves from tipping shoots of several clones indicate that the geometric method is at least as consistent as any of the others.

Moreover, considering that the youngest leaves which have not yet attained their maximum size, are likely to constitute only a small proportion of most normal populations of leaves for the assessment of areas, it would seem that the geometric method would be sufficiently reliable.

The range of clonal leaf shapes over which consistent and accurate determinations have proved possible is also impressive.

(*e*) A "leaf area grid" for tea leaves

Once a reliable mathematical expression was obtained for relating leaf area to length and breadth, a grid was designed for direct reading of leaf areas (Figure 4).

The method of use is as follows:

(1) The leaf is laid flat on the grid with its midrib along the axis AB, and such that the base of the petiole is at point A;

(2) The length of the leaf blade is read along the centimetre scale on AB;

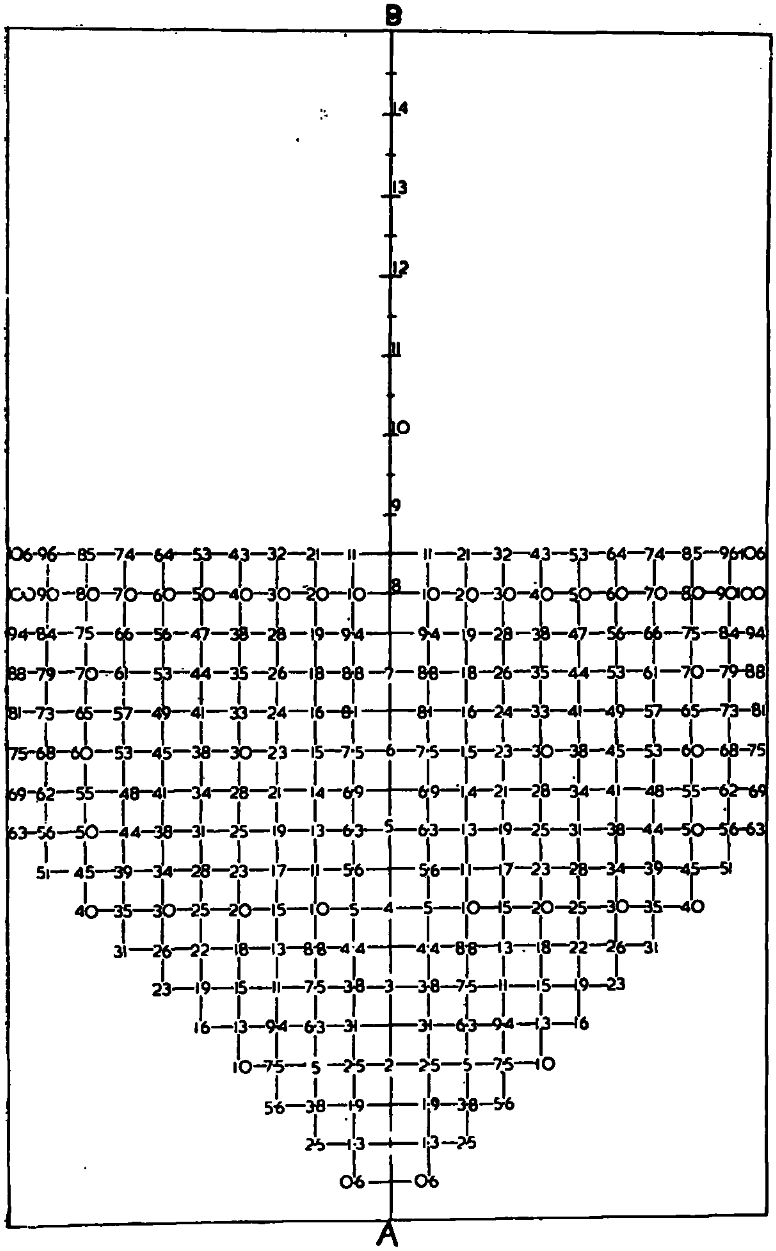

FIGURE 4—*The "leaf area grid" for use with leaves of tea*
(See text for details on method of use)

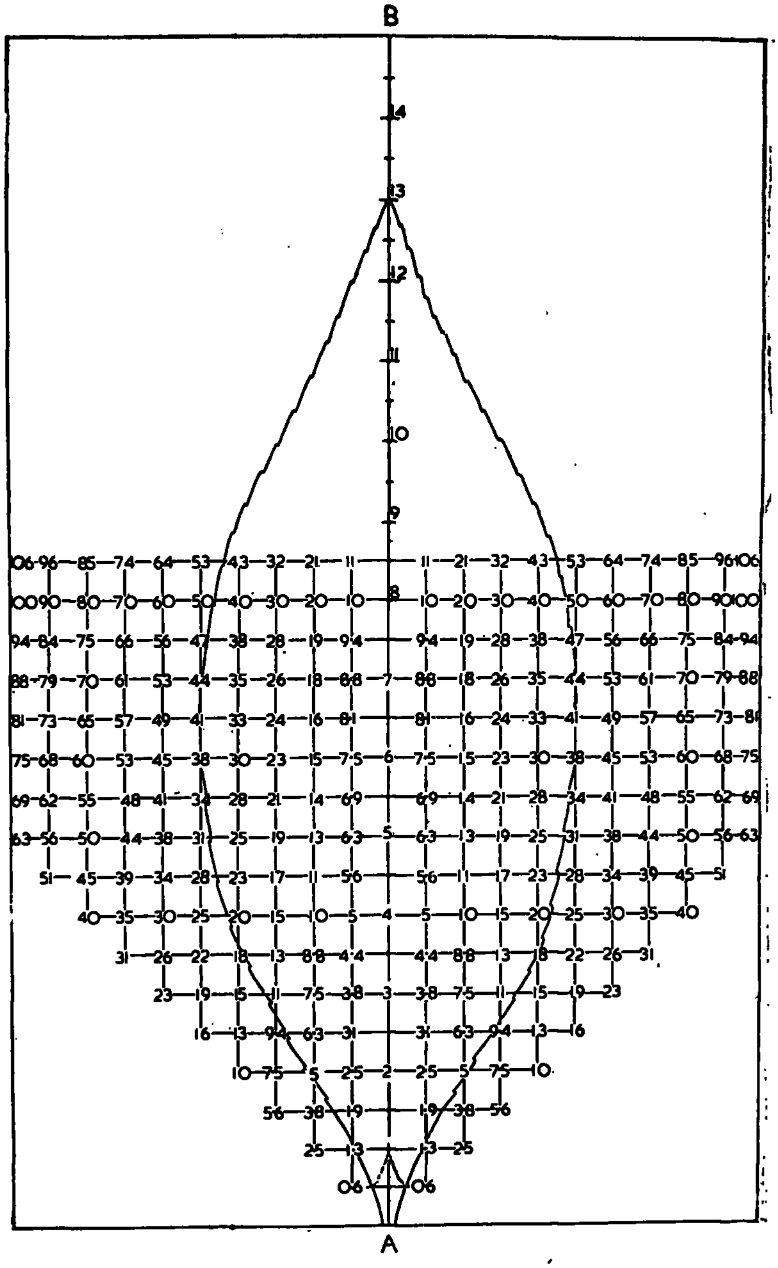

FIGURE 5—*Diagram of a leaf outline superposed to illustrate the method of use of the grid (See text for details)*

(3) Along a horizontal line corresponding to the middle point along the midrib, the value at the leaf margin is read on the scale. This point generally falls within the broadest part of the leaf;

(4) If the leaf is not perfectly symmetrical on either side of the midrib, it is necessary to either (*a*) read the values on both margins and obtain the mean, or (*b*) move the leaf slightly sideways to obtain the same reading on both sides;

(5) If the relevant point on the leaf margin does not fall exactly on an intersection, the value is estimated by taking into account the two figures along the horizontal grid lines between which the margin lies;

(6) In Figure 5 the outline of a leaf has been superposed to illustrate the method of use of the grid. The area would be read as follows :

(*a*) The length of the leaf along axis AB is 13 cm and the mid-point is at 6.5 along this axis

(*b*) Moving outwards horizontally at this point, the leaf margin lies at the intersection marked 41. Since the leaf is symmetrical, the readings are the same along both margins.

The area of the leaf is 41 sq cm.

In using the grid on mixed populations of tea leaves, peculiarities of leaf shape and size sometimes necessitate "estimation" of the areas. To evaluate the errors that may arise from this and other causes, a series of comparisons with standard methods has been carried out. Three such trials are summarised in Table 4.

TABLE 4

(*a*) *Estimation of the total area of a population of 225 leaves, representing ten different clones specially selected to cover a range of sizes and shapes*

		Total area in sq cm	Percentage of (ii)
(i)	Grid method	7046.0	98.7
(ii)	Punched disc method	7135.9	100.00
(iii)	Geometric method	6950.4	97.4

(*b*) *Total area of the leaves from four two-year old plants of clone WM 1/1. The determinations using the grid were carried out without detaching the leaves*

		Total area in sq cm	Percentage of (ii)
(i)	Grid method	1941.5	97.5
(ii)	Punched disc method	1992.0	100.0

(*c*) *Total area of 400 blueprint impressions consisting mainly of the first and second leaves of six different clones*

		Total area in sq cm	Percentage of (iii)
(i)	Grid method	5936.0	98.7
(ii)	Geometric method	6048.0	100.6
(iii)	Planimeter method	6012.0	100.0

It would appear that the grid yields estimates of leaf area differing by not more than 2-3% from methods hitherto employed. It caters adequately for a range of clonal leaf shapes and sizes (*a*) small, young leaves (*c*) and may be employed also on blueprint impressions (*c*). Accurate estimations are also possible on leaves still attached to plants (*b*).

With a little practice, the grid is very convenient and rapid to use and is well suited for measurements on relatively small plants. Transparent grids have been prepared on thick gauge photographic film for use in the field.

Summary

Most methods available for the estimation of leaf area require the detachment of leaves from the plant and so are destructive.

To allow for occasions when such defoliation is not possible, various devices have been employed to determine areas of leaves directly. One such is the "leaf area grid" which has been designed (Freeman & Bolas 1956) for use with apple and other leaves that have an approximately elliptical shape.

An expression relating leaf area to length and breadth measurements has been worked out. This appears widely applicable to a range of shapes and sizes of tea leaves. Utilizing this expression, a "leaf area grid" has been designed for use specially with tea.

The grid has proved to be convenient and reliable to use. Its special advantage is that it can be used on leaves while still attached to the plant and so is non-destructive, in contrast to several other methods which have hitherto been in use.

References

FREEMAN, G. H. & BOLAS, B. D. (1956) A method for the rapid determination of leaf areas in the field. *Rep. E. Malling Res. Sta.*—1955: 104-107.

THIRUMALACHARY, N. C. (1940) A rapid method of measurement of leaf areas of plants. *Indian J. agric. Sci.* **10:** 835-841.