THE ESTIMATION OF LEAF AREA IN FIELD CROPS

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(With One Text-figure)

The leaf area of a plant is a major determinant of its growth, for the new material produced by the plant in an interval of time is dependent on the size of its assimilating system. West *et al.* (4), and Gregory (2,3) have developed methods of growth analysis, using the rate of increase of dry matter per unit area of leaf ("Unit Leaf Rate" of West *et al.*; "Net Assimilation Rate" of Gregory) as a measure of the balance of the rates of assimilation and respiration. An analysis of plant growth in terms of this function and of the changes with time in leaf area provides more fundamental information than an analysis in terms of the relative growth rate.

In pot culture experiments, particularly in the early stages of growth, it is comparatively easy to measure leaf area directly, because the uniformity of the material allows of the use of a small number of plants. In the later stages, however, when the leaves become numerous and large, measuring the leaf area of every leaf may become extremely laborious. This difficulty is very much intensified in work on field crops. The great variability of the crop necessitates that all observations be made on a number of random samples, each consisting of many plants, in order that the growth changes and the magnitude of the experimental errors may be estimated accurately. The labour of measuring the leaf area of such large samples of plants directly would be impracticably great, for it would involve measuring separately several hundreds, possibly thousands, of leaves at each sampling time.

It is easy, however, to determine the mean leaf weight per plant, by cutting off and weighing the leaves of each sample, and dividing the total leaf weight by the number of plants. A high correlation exists between leaf area and leaf weight, and this fact has been utilized by Ballard & Petrie (1), who have used leaf weight instead of leaf area in calculating unit leaf rate. They point out, however, that other workers have found a drift in the leaf area : leaf weight ratio with time during

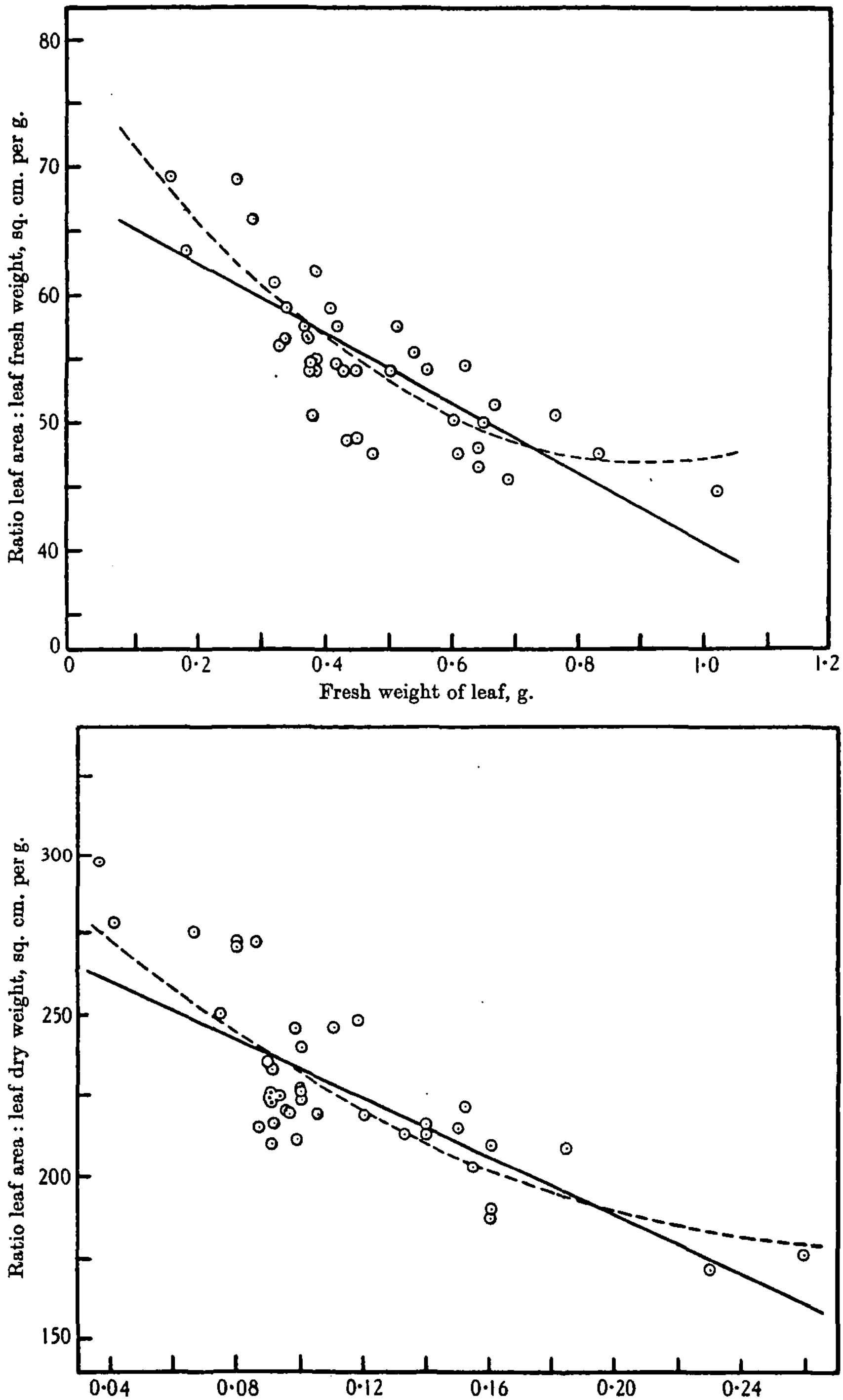

Fig. 1. Relation between leaf area : leaf weight ratio and leaf weight for single wheat leaves. Samples taken on 12th May, 1933.

the growing period, so that the relation between the unit leaf rate, as calculated by them, and as defined by West *et al.*, is not a simple one.

The correlation between leaf area and leaf weight may be made the basis of an indirect method of measuring leaf area. The procedure suggested in this paper for field crops is to estimate mean leaf weight from large random samples of plants, and to determine the leaf area : leaf weight ratio on quite small samples of single leaves. A similar method of estimating grain yield in cereal crops, by sampling for grain : straw ratio and weighing total produce has been suggested by Yates & Zacopanay (5). Gregory (3) has used an indirect method of estimating leaf area on the basis of the leaf area : leaf weight relation, in the later stages of a study of the growth of barley in pot culture, but gives no details of his procedure.

If the leaf area : leaf weight ratio were independent of leaf weight, it would be sufficient to estimate the mean leaf area per plant by multiplying the leaf area : leaf weight ratio, determined on the small sample of leaves, by the mean leaf weight per plant, obtained from the large sampling. On *a priori* grounds, however, it is obvious that the leaf area : leaf weight ratio must decrease with increasing leaf weight, for as the leaf grows in area it also increases in thickness.

Preliminary observations made on wheat leaves showed that there was, in fact, a negative regression of the leaf area : leaf weight ratio on leaf weight. The linear regression was found to account for 55 to 70 per cent of the variance of the ratio, and on fitting a second order term the additional reduction of the variance was small, ranging from 1 to 7 per cent. The nature of the relationship is shown in Fig. 1, where the leaf area per unit weight of leaf is plotted against leaf weight for forty wheat leaves, and the linear and second order regression lines are indicated. A similar type of relation holds whether fresh weight or dry weight is used, and whether single leaves or all the leaves of a shoot taken together are considered. In an experiment carried out on sugar-beet and mangolds, some of the results of which are considered later in this paper, negative linear regressions were found on all occasions, significant in 102 out of a total of 120 samplings. The mean reduction of the variance due to the regression was 71 per cent. It may be concluded that the linear regression is an adequate expression of the relationship between the leaf area : leaf weight ratio and leaf weight, and that the second order regression gives very little additional information to compensate for the large increase in the labour of computation, which fitting the extra term would involve.

METHOD OF ESTIMATING MEAN AREA PER LEAF AND MEAN LEAF AREA PER PLANT

Let A be the leaf area of a leaf of weight W, and let $\bar{A}$ and $\bar{W}$ be the mean values of A and W for the whole population of N leaves. Assuming that the relation between the leaf area : leaf weight ratio and leaf weight is linear, we have, apart from experimental errors,

$$\frac{A}{W}-\overline{\left(\frac{A}{W}\right)}=\beta\,(W-\bar{W})$$

or

$$\frac{A}{W}=\kappa+\beta W, \quad \text{where} \quad \kappa=\overline{\left(\frac{A}{W}\right)}-\beta\bar{W}$$

or

$$A=\kappa W+\beta W^2.$$

Making a summation over the whole population we have

$$S\,(A)=\kappa S\,(W)+\beta S\,(W^2). \qquad \text{......(1)}$$

Dividing by N we have

$$\bar{A}\ (\text{mean area per leaf})=\kappa\bar{W}+\beta\overline{W^2}. \qquad \text{......(2)}$$

If the mean number of leaves per plant for the whole population is $\bar{L}$, then the mean leaf area per plant $=\bar{A}.\bar{L}=\bar{L}\,[\kappa\bar{W}+\beta\overline{W^2}]$. $\bar{A}$, $\bar{W}$ and $\bar{L}$ are estimated accurately from a large random sampling but not $\overline{W^2}$, κ and β, since these involve the determination of individual leaf weights and areas.

Now $S\,(W^2)=S\,(W-\bar{W})^2+N\bar{W}^2$ and substituting in (1) we have

$$S\,(A)=\kappa S\,(W)+\beta N\bar{W}^2+\beta S\,(W-\bar{W})^2.$$

Dividing by N,

$$\bar{A}=\kappa\bar{W}+\beta\bar{W}^2+\frac{\beta S\,(W-\bar{W})^2}{N}. \qquad \text{......(3)}$$

In addition to the large sample, a small subsample of n leaves is taken, and the area and weight of each leaf is determined. From these areas and weights, k and b, estimates of κ and β respectively are calculated $\left(k=\overline{\left(\frac{a}{w}\right)}-b\bar{w}\right.$, where a and w are areas and weights of leaves of the subsample).

Now it is known that $\frac{1}{n-1}\,S\,(w-\bar{w})^2$ taken over the subsample is an unbiased estimate of $\frac{1}{N-1}\,S\,(W-\bar{W})^2$ taken over the large sample. Hence an unbiased estimate of $\frac{1}{N}\,S\,(W-\bar{W})^2$ is $\frac{N-1}{N}\cdot\frac{S\,(w-\bar{w})^2}{n-1}$, and since N is large (in the examples which follow $N=500$ to 1000), the factor $\frac{N-1}{N}$ may be replaced by 1.

Substituting in (3), we have

$$A_r \text{ (estimate of } \bar{A}) = k\overline{W} + b\overline{W}^2 + b\frac{S(w-\bar{w})^2}{n-1}, \qquad \ldots\ldots(4)$$

in which $\overline{W}$ is determined from the large sample, and k, b and $\frac{S(w-\bar{w})^2}{n-1}$ are obtained from the subsample.

It is important that the subsample be a strictly random selection from the whole population, in order that $\frac{S(w-\bar{w})^2}{n-1}$ may be an unbiased estimate of $\frac{S(W-\overline{W})^2}{N-1}$. If the subsample were to be used only for the estimation of the regression coefficient b, it would be preferable to select it to give the widest possible range of leaf weight so as to estimate b as accurately as possible, but this is not permissible if the subsample is also used to estimate the variance of mean leaf weight as well as b. A possible alternative method would be to take two subsamples, one selected to give a wide range of leaf weight to be used for the estimation of b, and the other, perhaps somewhat larger, a strictly random selection to provide an estimate of the variance of mean leaf weight. This method might be preferable if considerations of the work involved in measuring the leaf areas require that the subsample on which b is estimated be too small to give an accurate estimate of the variance.

It can be shown that the estimate of $\bar{A}$ obtained by this method is unbiased. From (4) the mean value of A_r from repeated subsamples of n leaves reduces to

$$\begin{aligned}\bar{A} &= \kappa\overline{W} + \beta\overline{W}^2 + \beta\sigma^2, \text{ where } \sigma^2 \text{ is the variance of leaf weight}\\ &= \kappa\overline{W} + \beta\overline{W}^2 + \beta(\overline{W^2} - \overline{W}^2)\\ &= \kappa\overline{W} + \beta\overline{W^2},\end{aligned}$$

which is the true value of $\bar{A}$ (expression (2) above).

Bias in other methods of estimating mean area per leaf

The mean area per leaf might be estimated from the product of the mean leaf weight $\overline{W}$, obtained from the large sample, and the mean leaf area : leaf weight ratio $\left(\frac{S\left(\frac{a}{w}\right)}{n}\right)$ calculated from the subsample.

It is obvious that the estimate of $\bar{A}$ obtained in this way must be positively biased, since the small leaves, which have a high leaf area : leaf weight ratio, will be overweighted, and the larger leaves, with smaller leaf area : leaf weight ratio, underweighted. The magnitude of the bias can be estimated as follows. For any leaf of the subsample of n leaves

$$\frac{a}{w}=\kappa+\beta w. \text{ Therefore } \overline{\left(\frac{a}{w}\right)}=\kappa+\beta\bar{w}$$

and the estimate of $\bar{A}=\overline{W}\times\overline{\left(\frac{a}{w}\right)}=\overline{W}\ (\kappa+\beta\bar{w})$.

The average estimate of $\bar{A}$ from repeated subsamples of n leaves

$$=\overline{W}\ (\kappa+\beta\overline{W})$$
$$=\kappa\overline{W}+\beta\overline{W^2}-\beta\sigma^2, \text{ since } \sigma^2=\overline{W^2}-\overline{W}^2.$$

The true value of $\bar{A}=\kappa\overline{W}+\beta\overline{W^2}$, so that the bias introduced is $-\beta\sigma^2$, which is positive since β is negative, and independent of the size of the subsample (n).

The objection to this method is partly mitigated if instead of the unweighted mean leaf area : leaf weight ratio $\dfrac{S\left(\frac{a}{w}\right)}{n}$, the weighted mean $\dfrac{S\,(a)}{S\,(w)}$ is used, so that $\bar{A}$ is estimated as $\overline{W}.\dfrac{S\,(a)}{S\,(w)}$. It can be shown, however, that this estimate is also positively biased, though to a less extent than the estimate from the unweighted mean. Thus, for any leaf of the subsample of n leaves

$$a=\kappa w+\beta w^2.$$

Therefore $$\bar{a}=\kappa\bar{w}+\beta\overline{w^2}.$$

The weighted mean leaf area : leaf weight ratio

$$\frac{S\,(a)}{S\,(w)}=\frac{\bar{a}}{\bar{w}}=\kappa+\beta\frac{\overline{w^2}}{\bar{w}},$$

and the estimate of $$\bar{A}=\overline{W}\times\frac{\bar{a}}{\bar{w}}=\kappa\overline{W}+\beta\frac{\overline{w^2}}{\bar{w}}.\overline{W}.$$

The mean value of the estimate of $\bar{A}$ from repeated subsamples of n leaves

$$=\kappa\overline{W}+\beta\overline{W}\left[\text{mean value of } \frac{\overline{w^2}}{\bar{w}}\right]$$
$$=\kappa\overline{W}+\beta\overline{W}\left[\text{mean value of } \bar{w}+\frac{S\,(w-\bar{w})^2}{n\bar{w}}\right] \qquad \ldots\ldots(5)$$

(since $\frac{\overline{w^2}}{\bar{w}} = \frac{S(w^2)}{n\bar{w}} = \frac{S(w-\bar{w})^2 + n\bar{w}^2}{n\bar{w}} = \frac{S(w-\bar{w})^2}{n\bar{w}} + \bar{w}$)

$$= \kappa\overline{W} + \beta\overline{W}\left(\overline{W} + \frac{n-1}{n} \cdot \frac{\sigma^2}{\overline{W}}\right) \text{ approximately,}$$

$$= \kappa\overline{W} + \beta(\overline{W}^2 + \sigma^2) - \frac{1}{n}\beta\sigma^2$$

$$+ \kappa\overline{W} + \beta\overline{W^2} - \frac{1}{n}\beta\sigma^2.$$

The bias introduced is therefore $-\frac{1}{n}\beta\sigma^2$, or $1/n$th of the bias in the estimate from the unweighted mean. It is dependent on the size of the subsample, becoming zero when n is very large, as obviously it must, since for the whole population $\overline{W} \cdot \frac{\bar{A}}{\overline{W}}$ is equal to $\bar{A}$. It should be pointed out that these estimates of the bias are correct only if the subsample is a random selection from the whole population, so that the mean of $\bar{w}$ tends to $\overline{W}$ in repeated subsamples of n leaves, and the estimate of σ^2, the variance of mean leaf weight, is unbiased.

Experimental results

A sampling experiment was carried out on sugar-beet and mangolds in 1934, in which growth observations were made by sampling at fortnightly intervals. The experiment consisted of six blocks, each of two plots, one of sugar-beet and one of mangolds, and the blocks were sown singly at successive intervals of a fortnight. Sampling was begun when the crops were thinned and ten complete samplings were carried out after the thinning of the last sown plots. On each occasion a random sample of twenty plants was taken from each plot, the number of leaves in the whole sample was counted, and the total fresh weight of leaf lamina determined. A random subsample of ten leaves was taken, one from each of ten plants selected at random from the twenty plants of a sample. The lamina was cut off and weighed and its area measured by printing on "blue-print" paper, cutting out the print and weighing it. The mean leaf area per plant was calculated for each sample by the three methods which have been discussed. The estimates obtained by the unweighted mean leaf area : leaf weight ratio method were consistently greater than those calculated by the regression method. The estimates from the weighted mean showed a similar but smaller positive bias. The mean values for the sixty samplings of sugar-beet and of mangolds are shown in Table I.

Table I. *Mean leaf area per plant, sq. dm. (mean of sixty samplings)*

Method of estimation	Sugar-beet	Mangolds
Unweighted mean	51·41	40·89
Weighted mean	40·81	30·49
Regression	38·03	28·97
Bias:		
Unweighted mean	13·38	11·92
Weighted mean	2·78	1·52
Ratio unweighted : weighted	4·8	7·8

It has been shown theoretically that the bias in the unweighted mean estimate should be n times the bias in the weighted mean estimate, where n is the number of leaves in the subsample. In this experiment $n=10$, and the ratio of the biases was found to be 4·8 for sugar-beet and 7·8 for mangolds. The latter figure is in fair agreement with theory, but the discrepancy for sugar-beet is more serious. The most probable explanation is that the estimate of mean leaf weight in the subsample was positively biased. In the sugar-beet there was a considerable development of axillary buds, forming many leaves of small area. In selecting the subsample of ten leaves, these were ignored, as it was difficult to devise any simple method of strict random selection which would include them, but they were included in the estimate of mean leaf weight in the large samples (W). The leaves on the main axis were selected by counting back from the youngest leaf in the order of production, until a leaf of a number selected from a table of random numbers was reached. In the mangolds, however, axillary leaves were rare. If $\bar{w}$ is an unbiased estimate of $\overline{W}$, it would be expected that in a series of samples, the number of occasions on which $\bar{w}$ exceeded $\overline{W}$ would be equal to the number on which $\bar{w}$ was less than $\overline{W}$. This was found to be true approximately for the mangolds, but for sugar-beet the number of occasions on which $\overline{W}$ was greater than $\bar{w}$ was markedly in excess of expectation. This is shown in Table II.

Table II

	Sugar-beet	Mangolds
Number of occasions on which $\bar{w}$ was greater than $\overline{W}$	40	28
Number of occasions on which $\bar{w}$ was less than $\overline{W}$	20	32

This bias in the estimate of $\bar{w}$ may have affected the estimates of mean leaf area by the regression method, for though k and b will not have been affected, the estimate of σ^2 may be biased.

The estimates of mean leaf area per plant calculated by the unweighted mean method were considerably more variable than estimates by the weighted mean, or regression methods. Table III shows the residual variances, after elimination of variance due to plots, times of sampling and the interactions with time of the linear regressions on sowing date.

Table III. *Residual variance of mean leaf area per plant* (72 D.F.)

Unweighted mean method	74·89
Weighted mean method	35·11
Regression method	48·14

The variances of the estimates calculated by the weighted mean and regression methods did not differ significantly.

A similar method of estimating mean leaf area per plant and per metre row of crop has been employed for wheat. The labour of cutting off and weighing the leaves from a large number of random samples, in order to determine the mean leaf weight $\overline{W}$, was found to be impracticably great, and to avoid this, the estimates were based on determinations of the leaf area: plant dry weight ratio and its linear regression on plant dry weight. The method of calculation was exactly similar to that described above, where the leaf area : leaf weight ratio and its regression on leaf weight were used. Significant negative regressions of the leaf area : plant dry weight ratio were found during the period from the beginning of May onwards, but during the earlier stages of growth the regression coefficients were small and not significant. Positive and negative values of the coefficient were equally frequent, indicating that during this period of growth the leaf area : plant dry weight ratio was independent of plant dry weight. The regression of the leaf area : leaf dry weight ratio on leaf dry weight was found to be consistently negative and significant on almost all occasions, as in the sugar-beet and mangold data.

Summary

1. It is shown that the leaf area : leaf weight ratio decreases with increasing leaf weight.

2. The relation between the leaf area : leaf weight ratio and leaf weight is well fitted by a linear regression equation.

3. A method of estimating the mean leaf area per leaf or per plant of a field crop by means of this regression is described. The mean weight per leaf is determined by a large sampling, and the leaf area : leaf weight ratio and its regression on leaf weight are estimated on a small subsidiary sample.

4. Alternative methods of estimation from the mean leaf weight and either the unweighted or the weighted mean leaf area : leaf weight ratio are shown to give positively biased estimates of mean leaf area.

5. It is emphasized that the small sample, from which the leaf area : leaf weight ratio and its regression on leaf weight are determined, must be a strictly random selection from the whole population.

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