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Costs of Measuring Leaf Area Index of Corn

by

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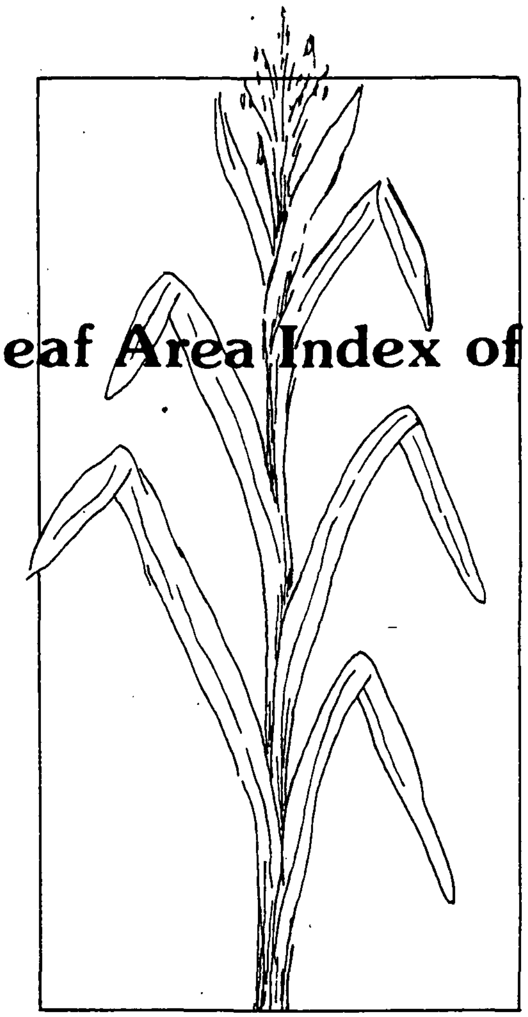

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16. Abstract

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ABSTRACT

Leaf area index (LAI) is an important biophysical descriptor of crop canopies. Accurate measurements of LAI are laborious and time-consuming. Many methods of measuring LAI of corn (Zea mays L.) have been reported and vary greatly in their accuracy, precision, bias, and ease of measurement. We examined the magnitude of plant-to-plant variability of leaf area of corn plants selected from uniform plots and evaluated four representative methods for measuring LAI. The number of plants required and the relative costs for each sampling method were calculated to detect 10, 20, and 50% differences in LAI using 0.05 and 0.01 tests of significance and a 90% probability of success ($\beta = 0.1$). The natural variability of leaf area per corn plant was nearly 10%. Additional variability or experimental error may be introduced by the measurement technique employed and by nonuniformity within the plot. Direct measurement of leaf area with an electronic area meter had the lowest CV, required that the fewest plants be sampled, but required approximately the same amount of time as the leaf area/weight ratio method to detect comparable differences. Indirect methods based on measurements of length and width of leaves required more plants but less total time than the direct method. Unless the coefficients for converting length and width to area are verified frequently, the indirect methods may be biased. When true differences in LAI among treatments exceed 50% of mean, all four methods are equal. The method of choice depends on the resources available, the differences to be detected, and what additional information, such as leaf weight or stalk weight, is also desired.

INTRODUCTION

Classical growth analysis studies and research on the interception of solar radiation by crop canopies both require frequent measurements of leaf area. Because accurate measurements of leaf area for crop canopies are laborious and time-consuming, numerous direct and indirect methods of measuring leaf area for various crops have been developed (5,6,9,11,14,16). The many methods reported in the literature have been summarized by reviewers (8,9,13) into at least 14 principal categories of methods which vary greatly in their precision, accuracy, and difficulty of performance. A researcher's choice of a method to measure leaf area depends largely on (i) morphological features of leaves to be measured, (ii) accuracy required, (iii) amount of material to be measured, and (iv) amount of time and equipment available.

If proper precautions are observed, many of the methods reported in the literature are sufficiently accurate for measuring leaf area of individual leaves and plants. In order to estimate leaf area index (LAI) of crop canopies, however, the variability in leaf area among plants within a plot must be considered as an additional source of experimental error. This inherent variability within crop canopies produces different estimates of LAI for the same treatment when more than one sample is acquired per treatment.

In practice, a researcher wants to know how many replications of each factor must be measured to be reasonably confident of detecting specific differences among crop canopies. He faces questions about how to allocate the finite number of measurements that can be acquired in a reasonable length of time between the number of measurements per plot and the total number plots (treatments) in the experiment. If he does not acquire enough samples or measurements per plot, his estimate of the true LAI of a plot will be too inaccurate to be useful. Conversely, he also wants to avoid taking more measurements per plot than is required to obtain an accurate estimate since such an approach would limit the number of plots that can be measured and possibly the scope of the experiment.

The first step is to decide how small a difference among treatments must be detected, or conversely, how large an error in LAI can be tolerated. This demands careful thinking about the use to be made of the estimates of LAI and about the consquences of a sizeable error. The figure finally reached may be quite arbitrary initially, but does represent a goal which may be refined as experience is gained.

In this paper we examined the magnitude of within plot errors for components of corn plants (Zea mays L.) selected from uniform plots and evaluated several methods for measuring LAI with known precision and probability of success. The approximate errors, the number of plants required, and the relative costs in time per sample for each method are also presented.

MATERIALS AND METHODS

Two field experiments were conducted on a Chalmers silty clay loam (fine-loamy, mixed, mesic Typic Arqiaquoll) at the Purdue University Agronomy Farm, West Lafayette, Indiana. In 1980 a single-cross corn hybrid ('Becks 65X') was planted on 22 May in 0.76 m wide rows and thinned to 50,000 plants/ha. Plants were sampled at growth stages V7, V10, V16, and R1 (12) according to the following procedure. From a randomly selected starting point in two different rows, 10 consecutive plants were sampled by cutting the plants at the soil line. Each plant was weighed immediately and separated into leaf blades (including exposed portions of leaves in the whorl), stalks (including leaf sheaths) and ears. The area of all leaves on a plant was measured with an optically scanning area meter (LI-COR model LI-3000 with conveyor belt). All plant parts were dried to constant weight at 75° C. Care was taken to minimize extraneous errors in stalk dry weights due to nonuniform drying by cutting the stalks into segments 20 to 30 cm long and by splitting each segment before drying.

In a second experiment conducted in 1982, corn was planted on 12 May in 0.76 m wide rows and thinned to 50,000 and 100,000 plants/ha. During the milk stage (R3) of grain development, 20 plants were randomly sampled from each plot as before. As each leaf was removed from the stalk, its length (L), maximum width (W), and area were measured. Leaf area was measured using area meter (LI-COR model LI-3100) and each leaf was dried to constant weight at 75° C.

Ratio of leaf area per unit leaf dry weight (specific leaf area, SLA) was calculated. Means, standard deviations, and coefficients of variation were calculated for each plant component.

The variation associated with directly measuring the area of all leaves on each plant with an area meter was assumed to represent the inherent variability in leaf area per plant with only a minimal contribution due to the measurement technique. Each of the other methods estimate leaf area indirectly and thus contribute additional uncertainty to the determination of leaf area. This additional uncertainty was estimated as the sum of squared deviations about a linear regression line (i.e., $(\hat{Y}_i - \bar{Y}_i)^2$, sum of squares for error, or SSE) (10). Leaf area per plant was regressed as a function of (i) the dry weight of leaves per plant, (ii) the sum of the product of leaf L and W for each plant, and (iii) the area of the largest leaf per plant. Regression coefficients were computed with ($Y = b_o + b_1X$) and without ($Y = b_1X$), an intercept term in the model using a General Linear Models program (7). Coefficients of determination (R^2) were calculated only for models with an intercept term. When the regression is forced through the origin, no correction for the mean is made and the sum of the deviations from the regression line is not zero (3,10). An R^2 calculated using these uncorrected sums of squares cannot be compared with a conventional R^2 that was computed with a corrected sum of squares. Both models can be evaluated by comparing the square roots of mean square error or the standard errors of the estimate (s_{yx}). An estimated total variance was calculated by summing the variance due to inherent variability of the leaf area per plant and the additional variance due to measurement technique (i.e., SSE). These estimates of total variation include errors due to within-plot variation and errors associated with the measurement technique.

The minimum number (n) of the basic sampling unit (i.e., one plant) required for a 90% probability ($\beta = 0.10$) of obtaining a significant result at the $\alpha = 0.05$ and 0.01 levels were estimated using the following equation (1,3):

$$n \geq 2(\sigma/\delta)^2(t_1+t_2)^2 \qquad [1]$$

where: n = number of replicates,
σ = true standard error per unit,
δ = true difference that it is desired to detect,
t_1 = significant value of t for the α level,
t_2 = value of t corresponding to the β level.

Since the value of n depends only on the ratio of σ/δ, coefficient of variation (CV) and percent difference (d) were substituted for σ and δ, respectively, in Eq. [1]. Because the number of degrees of freedom in t_1 and t_2 depends on n, initially n was assumed to be infinity and then adjusted in subsequent calculations until the smallest number of replicates that would satisfy the condition was determined (3). The average costs per plant in man-minutes for the four methods of measuring leaf area of corn plants were estimated from our experience and by interviewing other agronomists who have extensive experience in growth analysis research. Total costs for each method were calculated by multiplying the minimum of plants required to detect significant differences times the average cost per plant.

RESULTS AND DISCUSSION

Variation Among Plants

Means, standard deviations, and CV of several plant characteristics for the corn plants sampled are presented in Tables 1 and 2. CV normalizes standard deviations by the mean and is useful for comparing relative variations among stages of development and plant characteristics. The large variations in stalk weights among the plants sampled undoubtedly contributed to the large CVs for both total weights (Table 1). The largest CVs in total fresh and dry weights occurred prior to silking (Table 1) and are similar in magnitude to previously reported values for corn (4,15).

The CV of leaf area and leaf weight per plant decreased after silking when all leaves were fully expanded (Table 1). In most cases the CV (R1) for leaf area were smaller than CV for leaf weight (Table 1 and 2). CVs for specific leaf area (SLA), were much smaller than the CV of leaf area, and leaf, stalk, and total dry weights (Tables 1 and 2). The small CVs observed for SLA are consistent with the expected variances for ratio estimators when the components of the ratio are positively correlated (2,3,10). These ratios have lower variation than direct measures of area and mass and may be useful for estimating leaf area as based on area to weight ratios. It also appears feasible to estimate LAI using fresh weights with approximately the same accuracy as with dry weights. This assumes that moisture losses are minimized and plants are processed rapidly. Estimating LAI on a fresh weight basis has an additional advantage -- no fuel is required for drying large volumes of plant material with high moisture contents.

Methods of Measuring LAI

One question facing a researcher is how best to allocate finite resources to measure the leaf area of numerous plants and be reasonably confident of detecting significant differences among crop canopies. We selected four representative methods of measuring LAI to illustrate the advantages and disadvantages of single and multistage sampling methods. In

Table 1. Descriptive statistics for 20 corn plants sampled at four stages of development in 1980.

Plant Characteristic	Stage of Development†	Mean	S	CV
				%
Total Fresh Weight (g/plant)	1.75	118 a‡	21.8	18.5
	2.50	379 b	80.3	21.2
	4.00	880 c	116.2	13.2
	5.00	995 d	111.4	11.2
Total Dry Weight (g/plant)	1.75	11.3 a	2.1	19.0
	2.50	37.2 b	8.9	23.9
	4.00	95.4 c	12.6	13.2
	5.00	154.4 d	20.8	13.5
Stalk Dry Weight (g/plant)	1.75	5.2 a	1.3	25.3
	2.50	20.3 b	6.0	29.7
	4.00	62.0 c	9.0	14.5
	5.00	99.6 d	14.0	14.1
Leaf Dry Weight (g/plant)	1.75	6.1 a	1.5	16.3
	2.50	16.9 b	3.2	18.8
	4.00	33.4 c	4.1	12.2
	5.00	39.7 c	3.9	9.9
Leaf Area (m^2/plant) x 100	1.75	13.8 a	2.0	14.2
	2.50	36.1 b	5.8	16.0
	4.00	64.8 c	6.5	10.0
	5.00	66.0 c	4.5	6.8
SLA§ (m^2/kg) x 100	1.75	22.7 a	1.1	4.8
	2.50	21.6 a	1.2	5.7
	4.00	19.5ab	1.6	8.0
	5.00	16.7 b	1.1	6.3

† Stages of development are 7, 10, 16 leaves, and silking, respectively.

‡ Means of each plant characteristic followed by the same letter are not significantly different at α = 0.05 level of Duncan's multiple range test.

§ Specific leaf area = leaf area/leaf dry weight.

Table 2. Descriptive statistics for 40 corn plants sampled at milk-stage (R3) development in 1982.

Plant Characteristic	Plant Density	Mean	S	CV
	plants/m^2			%
Leaf Dry Weight (g/plant)	5	45.6 a†	7.4	16.2
	10	39.6 b	5.2	13.1
Leaf Area (m^2/plant) x 100	5	67.9 a	7.4	10.8
	10	69.2 a	5.5	7.9
Leaf Length x Width (m^2/plant)	5	94.0 a	10.8	11.5
	10	93.4 a	6.8	7.3
SLA‡ (m^2/kg) x 100	5	15.1 a	1.3	8.8
	10	17.6 b	1.7	9.4
LAF§	5	8.1a	0.66	8.1
	10	7.9a	0.33	4.1

† Means of each plant characteristic followed by the same letter are not significantly different at $\alpha = 0.05$.

‡ Specific leaf area = leaf area/leaf dry weight.

§ Leaf area factor = total leaf area/area of largest leaf on each plant.

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each of the methods presented below, plant density (plants/unit area of soil) also must be determined to calculate LAI. We have assumed that the errors in determining plant density are identical for each method and thus may be omitted for these comparisons.

In the first method (referred to as method I), the area of all leaves (A_L) on n plants is measured directly using a digital electronic area meter (e.g., LI-COR 3100). LAI is the mean leaf area per plant and is calculated as

$$LAI_1 = A_L/n. \qquad [2]$$

Areas of leaves with irregular margins or those with holes such as caused by insects can be measured by these devices (7). The errors of measurement with digital area meters are not always indicated but are probably less than 2% (6,8). For example, when the precision of the area meter was evaluated by repeatedly measuring (n=50) the area of a calibration plate, a soybean leaflet, and a corn leaf, the CV was 0.08, 0.17, and 0.34%, respectively. Leaves tend to fold and wrinkle slightly as they move between the rollers of the area meter, causing slight differences in the total area measured. These random errors of measurement associated with leaf area meter are very small compared to other sources of variation.

The second method (i.e.,method II) employs the relationship between leaf area and leaf weight of a subsample of leaves to convert the weight of a large sample of leaves into leaf area (8 9,16). Leaf area (A_L) and leaf weight (W_L) are measured on a subsample of leaves and total leaf weight, W_{TL}, only is measured on n plants. LAI is calculated as

$$LAI_2 = (A_L/W_L)(W_{TL}/n) \qquad [3]$$

This multistage sampling method uses a small number of plants to estimate specific leaf area which has a low CV and a larger number of plants to estimate total leaf dry weight which has a high CV (Tables 1 and 2). Variations in specific leaf area within one plot, plant, and leaf may exceed 10% (8,13) while variations in leaf dry weight per plant may exceed 20% (4,15,16).

In the third method (i.e., method III), area of each leaf on n plants is estimated as the product of leaf length (L), maximum leaf width (W), and a constant (b_1). LAI is calculated from the sum of these estimated leaf areas as

$$LAI_3 = \sum_{j=1}^{n} \sum_{i=1}^{m} (b_1 LW_i)_j / n \qquad [4]$$

where m is the number of leaves on the j^{th} plant, n is the number of plants sampled, LW_i is the product of length and width of i_{th} leaf, and b_i is an empirically derived area constant.

The general form of the relationship is $A = b_o + b_1 LW$, where b_o and b_1 are coefficients determined by regression that require checking if leaf shape changes with position on the plant or with plant age. Frequently b_o is not significantly different from 0.0 or is assumed to be 0.0 and the equation can be simplified (6). For example, leaf area of corn may be calculated by $A = 0.75$ LW. Reported values of b_1 range from 0.65 to 0.80 with b_1 generally increasing as LW increases (8,16) and changing with cultivar (6,8).

The magnitude of the plant to plant variability (CV) of the product of leaf length and width is approximately the same as for leaf area (Table 2). Plant density did not significantly affect the relationship of area to leaf LW. Area per leaf and the product of leaf length and width are plotted in Figure 1.

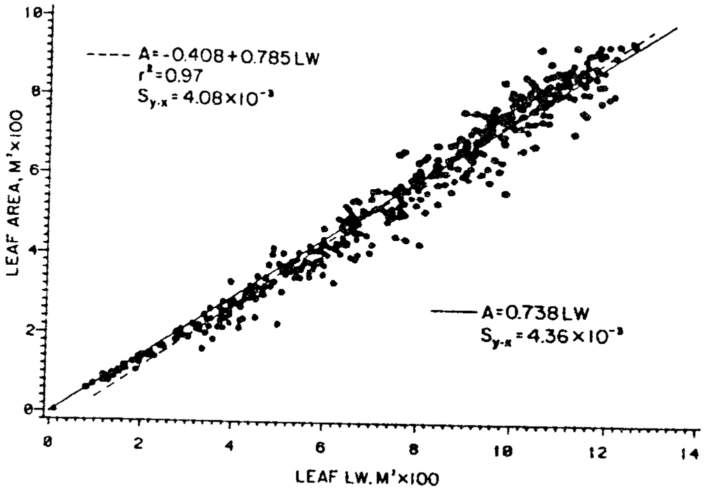

Fig. 1. Regressions and standard errors of the estimate (s_{yx}) for models of area per leaf of corn as functions of the product of leaf length and width in 1982 (n=496).

Although the intercept, b_o, was small, it was significantly (α = 0.01) different from 0.0. If b_o was assumed to be 0.0 anyway, the slope of relationship is 0.785. If the often reported (5,6,11,16) value of 0.75 had been used instead of the empirically-derived value of 0.785, the estimates of LAI for these data would be biased at least 4.1% low. An error of this size may be acceptable in some experiments but could produce further erroneous results if leaf shape changed due to treatment.

The fourth method (i.e., method IV) is an adaptation of the rapid or "short-cut" methods of estimating leaf area (5,11). A leaf area factor (LAF) is determined by measuring total leaf area for m plants in one replicate and dividing the total leaf area of each plant by the area of the largest leaf on that plant. Francis et al.(5) recommended using 10 plants to minimize errors in determining LAF for each genotype. In all other replicates only the area of the largest leaf per plant would be obtained for n plants and leaf area per plant would be calculated by using the LAF determined in the first replicate. Since the area of the largest leaf may be measured directly using a portable area meter or calculated using measurements of leaf L and W, this method could be nondestructive, using the same plants throughout the season. LAI is calculated as

$$LAI_4 = \sum_{i=1}^{n} (LAF)(b_1\ LW_{max})_i/n \qquad [5]$$

where: LAF is leaf area factor which is ratio of area of largest leaf to total leaf area per plant, LW_{max} is product of leaf length and width, and b_1 is the area constant.

Such a rapid method is designed primarily for use after silking when all leaves of corn are fully expanded. Prior to silking, LAF changes rapidly as new leaves emerge. A new LAF would have to be determined for each treatment on each sampling date. Furthermore the areas of leaves not fully expanded in the whorl of corn are probably over-estimated using linear measurements because the shape of immature leaves differs significantly from the shape of mature leaves. After silking, when all leaves are fully expanded, LAF should be relatively stable until the lower leaves begin to senesce.

Analysis of Costs

Data in Table 2 were used to illustrate the costs associated with measuring leaf area by these four methods. The mean CV over the two planting densities associated with directly measuring the area of all leaves on each plant using an area meter was 9.4% (Table 2) and represented a minimum inherent variability in leaf area per corn plant with only minor variation contributed by the measurement technique.

Each of the other methods indirectly estimated leaf area and thus contributed additional uncertainty to the measurement of leaf area. This additional variance is graphically ilustrated in Figures 2, 3, and 4 corresponding to the key variables used in methods II, III, and IV, respectively. In each figure, one line represents the best least squares fit of the model $Y = b_o + b_1X$. The second line is the least squares fit when the line is forced through the origin (i.e., $Y = b_1X$). The slope of the second line is simply the ratio of leaf area to each of the dependent variables. Forcing the line through the origin significantly increased (α = 0.05) the standard error of the estimate (i.e., s_{xy}) only for method II (Fig. 2). For this analysis of costs, we used the additional variance associated with forcing the line through the origin because ratio estimators are widely used (5,6,8,9,11,14,15,16). The mean CVs associated with measurement methods II, III, and IV were 14.7, 10.2, and 11.6%, respectively. Although CV will vary from experiment to experiment, the same relative ranking of CV for these methods of measuring LAI should be maintained.

The minimum number of replicates of the basic sampling unit (i.e., a plant) required for α = 0.05 and 0.01 are shown as functions of the CV and true difference of among treatments (Table 3). These data illustrate the value of a reduction in standard error per unit or CV. One cannot have a high probability of detecting a significant difference with any reasonable number of replicates unless the CV/d ratio (i.e., the σ/δ ratio of Eq. [1]) is 1.0 or less. Differences at least twice as large as the CV can be detected in most cases without excessive replication. For example, in order to detect a 10% difference in leaf area using α = 0.05 test of significance, at least 44 plants must be measured if the CV is 14.7% (i.e., method II). If the CV can be reduced to 9.4%, only 21 plants are required. Alternatively if the researcher is willing to gamble by accepting a 50% probability (β = 0.50) of obtaining a significant result, then 17 and 8 plants are required for CVs of 14.7 and 9.4%, respectively (1). Generally such a high probability of making a Type II error is bad from a researcher's view because one wants to make the correct decisions as frequently as possible and avoid losses of time and money on experiments with little chance of success.

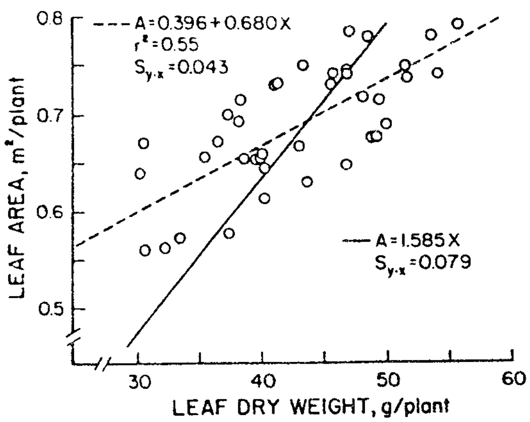

Fig. 2. Regressions and standard errors of the estimates (s_{yx}) for models of leaf area per plant as functions of the dry weight of leaves per plant in 1982 (n=40).

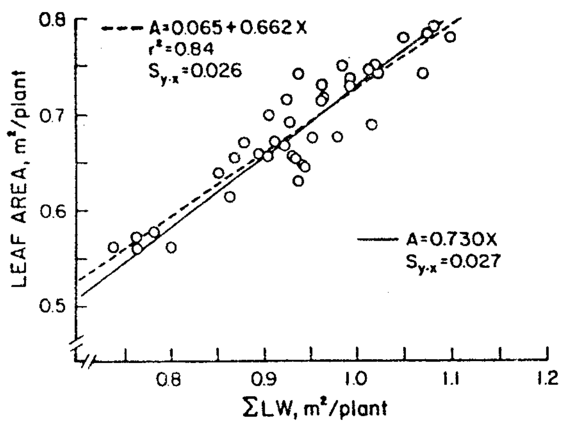

Fig. 3. Regressions and standard errors of the estimates (s_{yx}) for models of leaf area per plant as functions of the sum of the products of the length and width of leaves per plant in 1982 (n=40).

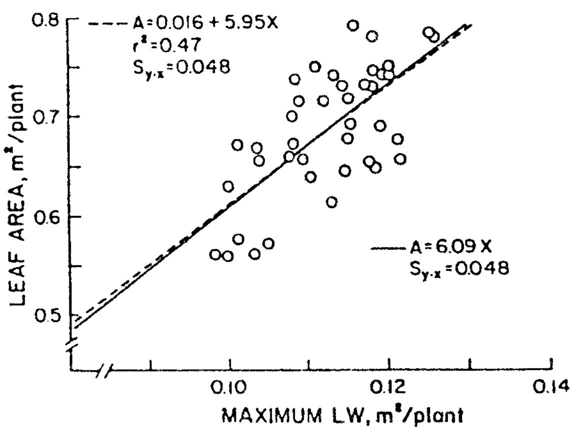

Fig. 4. Regressions and standard errors of the estimates (s_{yx}) for models of leaf area per plant as functions of the product of the length and width of largest leaf per plant in 1982 (n=40).

Table 3. Minimum number of plants required to detect true differences among treatments using $\alpha = 0.05$ and 0.01 tests of significance and 90% probability of success ($\beta = 0.1$).

		Test of Significance					
		$\alpha = 0.05$			$\alpha = 0.01$		
		True Difference, %					
Method†	CV	10	20	50	10	20	50
	%	number of plants					
I	9.4	21	7	2	29	10	3
II	14.7	44	13	4	63	18	5
III	10.2	23	7	2	32	10	3
IV	11.6	31	9	3	43	13	4

† Equations 2, 3, 4, and 5 are used to compute LAI for methods I, II, III, and IV, respectively.

In order to evaluate these four methods of measuring LAI, the average costs in time (i.e., man-minutes) were estimated for each step of a method (Table 4). Costs other than labor were not included in this analysis and it was assumed that the same skill level of labor was employed throughout. Destructive sampling was assumed for methods I and II and nondestructive sampling for methods III and IV. Nondestructive measurements may be repeated on the same plants; however, repeated handling and measuring the same plants may reduce their growth relative to undisturbed plants (13).

Total costs shown in Table 5 were calculated by multiplying the time required to acquire the necessary measurements on one plant (Table 4) by the minimum number of plants required (Table 3). For example, in order to detect 20% differences using method I the leaf area of at least seven plants must be measured which would require 56 man-minutes. The total costs for detecting 20% differences in leaf area are approximately the same for meth-

Table 4. Relative costs for measuring leaf area of one corn plant with 12 to 14 leaves. Equations 2, 3, 4, and 5 are used to compute LAI for methods, I, II, III, and IV, respectively.

Method	Activity	Time
		man-minutes/plant
I	Measure area of all leaves†	
	a. Harvest and transport	1
	b. Remove leaves	3
	c. Measure area	4
II	Measure area and weight of subsample of leaves‡	
	a. Harvest and transport	1
	b. Remove leaves	3
	c. Measure area	4
	d. Dry and weigh	1
	Measure weight of large sample of leaves§	
	a. Harvest and transport	1
	b. Remove leaves	2
	c. Dry and weigh	1
III	Measure length and width of all leaves	6
IV	Measure length and width of all leaves in one replicate¶	6
	Measure length and width of largest leaf in other replicates	1

† $Cost_1$ = n(8 min/plant)

‡ $Cost_2$ = (9 min/plant) + (n-1)(4 min/plant)

§ $Cost_3$ = n(6 min/plant)

¶ $Cost_4$ = (10/r)(6 min/plant) + (n)(1 min/plant), where r is replicate and n is number of plants on which only largest leaf is measured.

Table 5. Total relative costs for measuring leaf area of corn plants. Equations 2, 3, 4, and 5 are used to compute LAI for methods I, II, III, and IV, respectively.

		Test of Significance					
		α = 0.05			α = 0.01		
		True Difference, %					
Method	CV	10	20	50	10	20	50
	%	Man-Minutes					
I†	9.4	168	56	16	232	80	24
II‡	14.7	181	57	21	257	77	25
III§	10.2	138	42	12	192	60	18
IV¶	11.6	81	60	60	93	63	60

† $Cost_1$ = n(8 min/plant)

‡ $Cost_2$ = (9 min/plant) + (n-1)(4 min/plant)

§ $Cost_3$ = n(6 min/plant)

¶ $Cost_4$ = (10/r)(6 min/plant) + (n)(1 min/plant), where r is number of replicates (i.e., assumed that r = 4) and n is number of plants on which only largest leaf is measured.

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ods I and II (Table 5) even though the numbers of plants required nearly doubles (Table 3). Method IV requires the most plants (Table 3) but demands less time (Table 5) than method I to estimate leaf area accurately enough to detect true differences of 20% or less. One major assumption included in method IV is that costs of determining LAF for 10 plants per treatment in one replication can be distributed over four replications. Thus the total costs for method IV in Table 5 are 15 man-minutes plus the time required to measure only the largest leaf on n plants (Table 3). If an experiment has less than three replications, the cost advantage of method IV diminishes quickly. An additional hidden cost inherent in methods III and IV that is not included in these analyses is associated with determining the area coefficient (i.e., b_1 in Eqs. 3 and 4) which relates measurements of length and width to area. If the frequently cited area coefficient of 0.75 for corn is used rather than actually determined for each treatment, the estimates of leaf area may be biased (6). Likewise, if the LAF reported by Pearce et al. (11) is used without checking the data may be biased also. These biases may be acceptable if leaf shape does not change due to treatments and only relative estimates of LAI are required.

SUMMARY

In summary, many methods of measuring LAI have been developed which vary greatly in their accuracy and ease of measurement. The natural variability of leaf area per plant in a uniform field of corn may exceed 10% of the mean. Additional variability is introduced by methods which estimate leaf area based on area to weight ratios or measurements of leaf length and width. Direct measurement of leaf area (method I) had the lowest CV, required the fewest plants, but required approximately the same amount of time as the leaf area/leaf weight ratio method (method II) to detect comparable differences. Indirect methods of estimating leaf area based on measurements of length and width of leaves (i.e., methods III and IV) required more plants but demanded less total time than the direct method. However, these indirect methods may be biased unless the area coefficients are verified frequently. When the true differences in LAI exceed 50%, all methods require approximately the same amount of time. The method of choice depends on the resources available, the differences to be detected, and what additional information such as leaf weight or stalk weight is also desired. Efficient and creative multistage sampling schemes can minimize experimental error and cost.

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