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## ABSTRACT

Leaf area is measured by several methods and area per unit leaf weight (specific leaf area) is calculated. Leaf area index is then calculated as the product of leaf yield and specific leaf area. Procedures and equipment needed are described. An equivalent time of 6 class hours will be required for either a college junior-senior or graduate-level course. Students should learn to select the most acceptable method of leaf area determination for a given species and to evaluate management systems based on leaf area index concepts.

Additional index words: Alfalfa, Reed Canarygrass, Specific Leaf Weight.

There is a need within the plant sciences to develop student competence in interrelating basic principles for problem solving. It is the purpose of this article to provide teaching resources that apply selected scientific principles to the study of plant growth. Some background information will be presented, as well as a description of supplies and equipment needed.

# **IMPORTANT PRINCIPLES**

Watson (13) was among the first to use the concept of leaf area index (LAI) for describing the complicated interrelationships of plant growth capabilities and light interception. Leaf area index refers to the leaf area (one side) per unit area of land. The importance of this unit of measure is in relation to interception of light for maximum growth. By definition, any LAI below 1 will allow some light energy to fall onto the soil. Due to the natural display of leaves, however, the LAI must be considerably above 1 before most of the light will be intercepted.

Grazing management of plants should be based on their morphology and leaf area. Small prostrate plants that are difficult to defoliate may be grazed continuously and yet retain adequate leaf area for growth. Tall growing plants that are easily defoliated should be grazed quickly and at infrequent intervals with incomplete leaf removal. If some leaf area remains, regrowth will be fast and will not depend entirely on food reserves. The suitable objective could be to control leaf removal by grazing or harvesting to maintain an optimum LAI for maximum yields. Several reviews are available on the leaf area index concept (1, 3, 4, 12).

Leaf area index is the product of yield  $(g m^{-2}) \times \%$ leaves X specific leaf area  $(m^2 g^{-1})$  and is a dimensionless number. The percent leaves is measured by separating leaf and nonleaf tissue from a representative subsample of the herbage harvested for yield. The leaf portion should include only major light-intercepting plant parts. Generally, petioles or sheaths of the leaf are not included as leaf tissue. Specific leaf area (SLA) is the ratio of leaf area to leaf weight. Specific leaf weight (SLW) is reciprocal of (SLA) and is used by some workers to relate such effects as shade, age, and leaf position in the canopy (7, 9).

Many methods have been used for determining the area of leaves and each researcher develops a method that works best with a given crop and facility available. The methods used in this exercise include mechanical planimeter, photoplanimeter (5), weight of image (2), and length by width measurements (4). Other methods have been devised, including resistance to air flow (6, 8), and an electronic instrument for the nondestructive measurement of leaf area, leaf width, and leaf length.<sup>3</sup> A commercially available area meter accumulates electronic measurements as the leaves pass along a transparent conveyor.<sup>4</sup> The instrument is costly but very versatile and has a high degree of accuracy.

Given a field plot and the necessary equipment, the student will be able to (i) determine leaf area by several methods, (ii) select the most acceptable method (of those used) for the given species, and (iii) state the importance of leaf area index in studying crop growth.

## MATERIALS AND METHODS

The LAI and SLA will be measured for crops with compound and simple leaves. Alfalfa (*Medicago sativa* L.) and reed canarygrass (*Phalaris arundinacea* L.) are used as examples here. Facilities needed are quadrats, paper bags, scissors, drying oven, analytical balance, millimeter ruler, mechanical planimeter, and photoplanimeter. The sequence of events is illustrated in Fig. 1.

#### Step 1

Harvest samples and determine leaf-stem ratio. Four uniform areas for each species will be selected at random as replications from solid stands. A land area of  $1 \text{ m}^2$  will be marked by a quadrat for each replication.

Grass Stand (Reed Canarygrass). For each replication, harvest the growth inside the quadrat. Select 10 tillers as a subsample from each replication for determination of leaf-stem ratio. Remove leaf blades from tillers. Select four random blades (about 25  $cm^2$ ) for leaf area measurement as described in Step 2 below. Dry and weigh remaining herbage from area harvested. Dry and weigh plant portions separated in the subsample. These data will be used to obtain percentage of leaf blade and weight of leaves per unit area. Example data are in Table 1.

Legume Stand (Alfalfa). Harvest herbage as with the grass. Select 10 stems at random for determination of leaf-stem ratio. Separate leaflets from stem. Select eight or more leaflets (about 35cm<sup>2</sup>) for leaf area measurement as described in Step 2. Dry and weigh leaflets and stems separated in subsamples.

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<sup>&</sup>lt;sup>3</sup>Hollister, A. L., and M. D. Clegg. 1970. A portable instrument for the nondestructive measurement of leaf area. Agronomy Abstr. p. 80.

p. 80. <sup>4</sup>Model AAM Automatic Area Meter. Distribution by Far East Mercantile Corp. Room No. 1201, 50 East 42nd St., New York, N. Y. 10017.

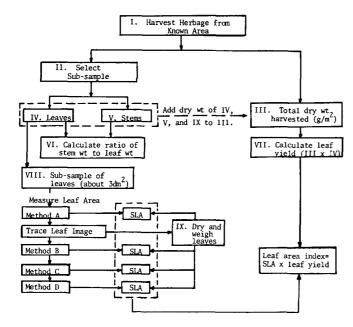


Fig. 1—Flow diagram for determination of leaf area by four methods and for calculation of leaf area index. Leaf material is considered as major light-intercepting tissue such as leaflets and leaf leaf blades.

### Step 2

Measure leaf area. The same leaf subsample will be used to determine the area by all four methods.

Method A. Place leaves on photoplanimeter and determine area (column A, Table 2).

The principle of area measurement with a photoplanimeter considers light from a fixed area impinging on a photo cell. With no leaves present the output of the cell is attenuated to create a maximum reading on a microampere meter. Any leaf material covering the fixed area will cause a linear reduction of output from the photo cell as read from the meter. The photoplanimeter can be constructed inexpensively from easily obtained parts (Fig. 2). The description that follows is a simplified version of other devices (5). The basic unit is a wooden box with bottom portion of 25 by 25 by 40 cm and a hinged lid portion of 25 by 25 by 18 cm. A 25watt red incandescent lamp is placed inside the bottom portion of the box and is connected to a rheostat. The box should be painted

Table 1-Herbage yield, percent leaves, leaf weight per unit land area, and leaf area index of reed canarygrass and alfalfa.

	Yield <b>;</b> g m <sup>-2</sup>	Subsample wt., g			%	Leaf weight,†	
Rep.		Leaf	Stem	Total	leaves	g m <sup>-2</sup>	LAI‡
_		1	Reed ca	narygra	55		
1	80	6.64	1.45	8,09	82	66 64	2,06 1,85
2 3	71 77	8.92 4.52	0.99 0.73	9, 91 5, 25	90 86	66	1.95
4	70	6.39	0.71	7,10	90	63	1,89
Avg	74				87		1.94
			Al	falfa			
1	113	1,63	1.84	3.47	47	53	1,52
2	131	1,16	1,32	2,48	47	61	1,98
3	97	1,52	1,20	2,72	56	54	2.15
4	88	1,21	1,13	2.34	52	45	1.52
Avg	108				50		1,79

\* Include weight of subsample.  $\dagger$  Product of % leaves and herbage yield.  $\ddagger$  Product of SLA from Table 2 and weight of leaves per m<sup>2</sup>.

Table 2-Leaf area (cm<sup>2</sup>) and leaf weight (g) of subsample used to determine leaf area per gram of leaf (SLA) as determined by several methods. All four measurements made on same subsample,

-	Rep.	Method of measurement*					Leaf weight	SLA.
Species		A	в	С	D	Avg†		m <sup>2</sup> g <sup>-1</sup>
Reed canary- grass	1 2 3 4	36.5 36.5 42.2 40.0	37,5 36,7 40,9 40,9	37.4 38.2 43.4 41.4	39.9 37.1 42.5 41.1	37.8 39.4 42.2 40.8	.121 .117 .142 .136	.0313 .0289 .0296 .0301
	Avg	38,8	39,0	40.1	40.1			.0298
Correl. coeff.‡		. 93	1,00	. 92	. 89			
Alfalfa	1 2 3 4	33,2 28,3 30,6 29,8	31.3 28.6 31.0 30.4	46.4 40.4 44.2 42.9	30.2 27.9 31.9 29.6	31.5 28.2 31.1 29.9	.110 .088 .078 .090	.0286 .0322 .0396 .0332
	Avg	30,5	30,3	43.5	29.9			.0334
Correl. coeff.		<b>.</b> 86	1,00	. 96	.84			

\* A-photoplanimeter; B-mechanical planimeter; C-length  $\times$  width of leaf portion; D-weight of image.  $\dagger$  Length  $\times$  width not included in average for alfalfa. Correction applied should be true area/length  $\times$  width; (30.3/43, 5 = .71).  $\ddagger$  Correlation coefficients are based on method B as calculated in Table 3.

black on the inside and have a 75- by 75-mm hole in the top. A glass plate placed over the leaves will hold them in position. A selenium photo cell<sup>5</sup> (83 by 83 mm) is mounted in the lid of the box. The cell is directly over the opening in the base when the lid is closed. The photo cell is connected at a 15-µampere meter. A switch in one of the microampere meter wires is used to disconnect the cell output whenever the lid is lifted and protects the meter from a surge of power. A 1000-ohm variable resistor is placed parallel with the meter. Steps for measurements are: (i) Adjust meter to read maximum by regulating the voltage (by use of rheostat) to the lamp. Fine adjustments are made with the variable resistor. The switch between the photo cell and the meter should open whenever the lid is lifted. With light off, meter should read 0. (ii) Place leaves on the glass plate that covers the opening in the base and the reduced light due to leaves results in reduced meter reading. (iii) Read the meter. The area of leaves on the planimeter is proportional to the reduced reading. A calibration curve should be made for the instrument for leaves of each species used to relate meter reduction to leaf area. (iv) Calibrate by placing varying amounts of leaves on the planimeter. The area of these leaves can be obtained with the mechanical planimeter and plotted against meter reading. At the time of calibration a known area of black paper should also be measured with the same procedures as for the leaves. The stability of the planimeter can be checked prior to using it by using this standard paper.

Method B. Now place leaf blades or leaflets on thin paper and trace the image; a photocopier can be used if available. Dry and weigh the leaves used for leaf area (weights in Table 2). Using a mechanical planimeter, measure the leaves from traced images and enter area in column B of Table 2. The mechanical planimeter is available from most engineering supply outlets and is often used as the standard method for determining area. It is time-consuming and requires some patience for high accuracy.

Method C. Length and width measurements should be taken before cutting the images from the copy paper. The sum of the products of length and width is an estimate of leaf area (column C, Table 2). The leaf dimension method of determining leaf area requires only a millimeter rule, but it is also time-consuming. Various treatments of length and width measurements have been used for many species (2, 7, 11, 14). As an example, for cotton (Gossypium hirsutum L.) Wendt (14) found that a relationship existed between the log of leaf area and the log of leaf length,

<sup>&</sup>lt;sup>5</sup> Photocell available from Internation Rectifier, 233 Kansas St., El Segundo, California (Catalogue No. B30PL, P2). Other supplies available from any radio supply company.

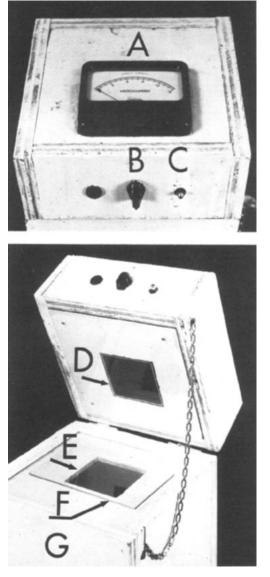


Fig. 2—Photoplanimeter. Upper, shows meter and lid in closed position for measurement. Lower, shows lid in position during placement of leaves and box containing light source. (A) 15 μamp meter; (B) 1000-ohm variable resistor (connected in series with meter to photocell); (C) Switch (in one line between photocell and resistor); (D) Selenium photocell connected to meter; (E) Opening to light source; (F) Glass plate to secure leaves; (G) Box containing light source (23- by 23- by 46-cm outside dimensions).

while Johnson (7) found that the squared length (to the side of the main vein) was proportional to leaf area. A conversion factor for each type of leaf can be determined by measuring the dimensions for leaves with known areas. The area of alfalfa leaves was found to equal the length X width X a factor of 0.71. The area of reed canarygrass leaves equaled the length X width measurement and no correction was needed, since only center sections having parallel edges were used.

Method D. Now cut and weigh the images from the copy paper. Make a standard ( $cm^2$  per mg of paper) using weights of known areas of a similar paper (2). The product of image weights and area per unit weight of copy paper gives leaf area of subsample (column D, Table 2).

## Step 3

Summarize LAI data. Data as in Table 2 include measurements of leaf area and leaf weight of each subsample. Specific leaf area

will be calculated using subsample area averaged over four methods. (i) Calculate the leaf area per unit leaf weight (SLA) by dividing the area by the weight of leaf area subsample. (ii) Calculate percentage of leaves (Table 1 from weights of leaves and stems). (iii) Find the weight of leaves per unit land area (Table 1) as the product of percent leaves and total yield. (iv) Calculate the area of leaves per unit land area (LAI) as the product of SLA and weight of leaves per unit land area.

## DISCUSSION AND INTERPRETATION

Selection of a method for determining the area of irregular leaves will depend on the ability of the operator to obtain repeatable results or low error. Also, the measurements must provide absolute values or have some correction factor applied to give absolute values. All methods used in this study provided acceptable absolute values, except for length-by-width measurements of the broadleaf species. The correction factor is the ratio of method C to method B, where method B (mechanical planimeter) is considered as the standard. Correlation coefficients will indicate how any one method compares with the standard (10). A correlation coefficient of .92 indicates that 85%  $(.92 \times .92 \times 100)$  of the variability between replications is due to different amounts of leaf area and 15% is due to factors such as inaccuracies in the method and to operator errors. Correlation coefficients can be calculated as outlined in Table 3. Proceed by entering measurements from two methods to be compared in columns 1 and 2 (X and Y, respectively, in Table 3). Find mean of both X and Y. Enter deviation of each observed value from mean of X in column 3. Do the same for Y observations and enter in column 4. Square the deviations and enter in appropriate columns (5 and 6). Find cross product of x and y deviations and enter in column 7. Sum all four products of each deviation (bottom of column 7). Substitute proper values into the formula at bottom of Table 3 and solve to find correlation coefficient.

Many substitutions may be made in the procedures, species selected, and leaf area determination methods compared. Leaf area index accumulation could be followed over time. Leaf area could be determined with only one method and the correlation calculations could be ommitted. When more than one method is used, however, students should be expected to select the most acceptable method from among those used. The students should be required to read references concerning the concept of leaf

Table 3–Calculations for correlating two methods of measuring leaf area; mechanical planimeter (X) and length  $\times$  width (Y). Data are for reed canarygrass from Table 2. Repeat for each possible comparison with method B.

		rement thod	Deviation from mean		Product of deviation		
Rep.	x	Y	x	у	x <sup>2</sup>	y²	xy
	[1]	[2]	[3]	[4]	[5]	[6]	[7]
1	37,5	37,4	-1.5	-2.7	2,25	7.29	4.05
2	36.7	38.2	-2.3	-1.9	5.29	3.61	4.37
3	40.9	43.4	+1.9	+3,3	3.61	10.89	6.27
4	40.9	41.4	+1.9	+1.3	3,61	1.69	2.47
Sum	156.0	160,4	0.0	0.0	14.76	23.48	17.16
Mean	39.0	40,1	0.0	0.0			
Correlat	ion	Sum x	<u>y</u> =	17	7.16	- 02	

coefficient  $\sqrt{(\text{Sum x}^2)(\text{Sum y}^2)} = \sqrt{(14.76)(23.48)} = .92$ 

area index and measurement of leaf area. A report should be written using the form of a scientific paper and should include a discussion of the practical applications of LAI.

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