

## 4. Direct Measurements of Canopy Structure

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### I. INTRODUCTION

Classical growth analysis studies and research on the interception and utilization of light by plant canopies require frequent measurements of canopy structure including the amount and organization of the above ground plant material. The location and orientation of each piece of foliage is also a key input to models of the radiation scattering characteristics of canopies at both optical and microwave wavelengths. Because accurate direct measurements of plant canopy structure are laborious and time-consuming, numerous methods of measuring foliage area and foliage angles have been developed. These methods vary greatly in their precision, accuracy, and difficulty of performance. The method of choice depends largely on (i) morphological features of foliage elements to be measured, (ii) accuracy required, (iii) amount of vegetative material to be sampled, and (iv) amount of time and equipment available.

While this chapter presents an overview of the principal methods for directly estimating the size and orientation of all components of the canopy, it focuses on measuring the areas and orientations of leaves (Sections II and V, respectively). Methods of measuring the surface areas and the masses of various plant parts are briefly discussed in Sections III and IV, respectively. Before a researcher can apply any of these methods, he must determine the number of samples required to be reasonably confident of detecting specific differences in the structures of plant canopies. Therefore, statistical determinations of the number of samples needed are also briefly reviewed in Section VI.

### II. LEAF AREA AND LEAF AREA INDEX

Leaf area ( $A_L$ ) is the area of one side of green leaves per plant and leaf area index (LAI) is the leaf area per unit area of soil surface (Kvet and Marshall, 1971; Ross, 1981). Thus  $A_L$  and LAI denote one half of the total leaf surface area of broadleaf plants. This definition implies that a leaf receives light mainly from one direction

and is appropriate for most broadleaf plants and grasses; however, for conifers and plants with spirally twisted or cylindrical (e.g., onions) leaves the total area of the assimilatory surface may be appropriate. In any case the meaning of terms, such as leaf area, foliage area, or assimilating surface, should be clearly stated.

Petioles are physiologically closer to stems than to leaves and generally leaf area refers to lamina area only. Stipules and cotyledons are frequently included with the true leaves. In grasses the leaf sheath usually forms part of the stem surface and is calculated accordingly. The method of estimating the surface area of inflorescences and other 3-dimensional plant parts must be selected in each case. In general the areas of these organs are small relative to the area of leaves, but this should be verified for each species.

Direct methods of measuring leaf area may be divided into: (a) leaf tracing methods, (b) methods based on matching of standard leaf shapes and sizes, (c) calculation methods based on linear measurements, (d) methods based on leaf area to mass relationship, and (e) optical planimetric methods. Each method is briefly described and its principal advantages and disadvantages are discussed.

#### **A. Leaf Tracing Methods**

The contour of a leaf is drawn on graph paper and its area is measured by counting the squares or dots within the leaf outline (Kvet and Marshall, 1971). Photocopies of leaves could also be used. Alternatively the leaf outline may be cut out, weighed, and area calculated based on an area to weight ratio for the paper. This is one of the earliest methods for determining leaf area and has been used extensively to calibrate all other methods.

The chief advantages of this method are its simplicity, and its reasonable precision and accuracy, if executed with care. Errors for measuring  $A_L$  are typically less than 1%. This technique can be implemented without elaborate equipment. The efficiency of this method is low, that is much time is required to determine the area of each leaf. It is also poorly suited for crinkled or small compound leaves.

#### **B. Methods Based on Matching of Standard Leaf Shapes and Sizes**

Sets of standard leaves or outlines of leaves with known areas are assembled for each species. Leaves from the test plants are matched with the reference shapes and their areas are recorded as that of the reference shape most closely resembled. This method is efficient, simple to use, and requires no special equipment. It is also nondestructive and the same plants can be measured repeatedly as they develop. The precision varies with the operator but errors for measuring  $A_L$  of less than 10% are typical for an operator with experience (Kvet and Marshall, 1971). However the preparation of complete sets of leaf sizes and shapes for each species can be time consuming. The American Phytopathological Society (3340 Pilot Knob Road, St. Paul MN 55121, USA) has produced several assessment keys for various plant diseases which may be instructive for preparing keys for leaf sizes and shapes.

One variation of the technique is to determine mean area per leaf by any convenient method and then count all of the leaves on the plant. Once the mean area per leaf is established with an acceptable confidence interval, this method is very rapid and efficient. However in some plants leaf size changes as the plant develops or changes from plant to plant and thus the standard deviation of the mean area per leaf may be unacceptably high.

### C. Calculation Methods Based on Linear Measurements

The leaf is modeled as a simple geometric shape and the area ( $A_L$ ) is determined by its linear dimensions, i.e., length ( $L$ ) and maximum width ( $W$ ) using the formula:

$$A_L = b_1 L W \quad (1)$$

where  $b_1$  is the regression coefficient. The value of  $b_1$  for a triangle is 0.5 and the closer the coincidence of the shape of the leaf is to a rectangle, the nearer  $b_1$  is to 1.0. The value of  $b_1$  for many grasses, e.g., corn and wheat, is approximately 0.75, while  $b_1$  for broadleaf plants, e.g., cotton and sugarbeet is approximately 0.65. Kvet and Marshall (1971) list typical values of  $b_1$  for selected crop and forest species.

This method is efficient and relatively simple to implement. Only a ruler, pencil, and paper are required, although a caliper or recording meter stick (Arkebauer and Norman, 1989) certainly increases efficiency and convenience. Measurements of length and width are nondestructive and the same plant can be measured repeatedly.

The accuracy of this method varies because the shape coefficient ( $b_1$ ) may change considerably with cultivar, developmental stage, and growing conditions (Kvet and Marshall, 1971; Ross, 1981). Thus  $b_1$  may require repeated determinations for each stand.

In one variation of the technique the area of the largest leaf is determined on the plant and then its area is correlated with the total leaf area of the plant (Pearce *et al.*, 1975). The leaf area factor should be determined using at least 10 plants for each genotype to minimize errors. This short-cut method is most suitable for gramineous plants (e.g., maize, wheat, sorghum) after all leaves are fully expanded. Prior to full leaf expansion, the leaf area factor changes rapidly as new leaves emerge. A new leaf area factor would have to be determined for each date. After anthesis, when all leaves are fully expanded, the leaf area factor is relatively stable until the lower leaves begin to senesce. This short-cut method is very rapid and nondestructive. It is particularly suited for replicated experiments where relative differences among treatments is an important factor (Daughtry and Hollinger, 1984).

### D. Methods Based on the Leaf Area to Leaf Mass Relationship

These methods employ the relationship between leaf area and leaf mass of a representative subsample of leaves to convert the mass of a large sample of leaves into leaf area ( $A_L$ ). Leaf area ( $A_s$ ) and leaf mass ( $M_s$ ) are measured on a small

subsample of leaves and total leaf mass ( $M_L$ ) only is measured for a larger sample of leaves. The representative subsample of leaves may be all of the leaves from a few plants so that all ages and sizes of leaves on plants are included. Alternatively, all of the leaves for the total leaf mass sample could be mixed together and one or more small random subsamples leaves could be selected and measured.

$$A_L = (A_s/M_s)M_L \quad (2)$$

This multistage sampling method uses a small sample of leaves to estimate specific leaf area (SLA = area/mass), which has a relatively low coefficient of variation (CV), and a larger sample of leaves to estimate total leaf mass per unit area of soil, which has a relatively high CV (Daughtry and Hollinger, 1984). Thus resources are focused on the source of the largest variance. However, the additional step of estimating leaf area from leaf mass does increase the overall CV for the estimate of leaf area compared with the tracing and optical planimetric methods. The question the researcher must address is whether the gain in efficiency of this technique sufficiently offsets the increase in overall error.

To be most efficient, this method assumes that the leaf area to leaf mass ratio (SLA) is constant. In fact, SLA changes with genotype, stage of development, and environmental conditions and thus SLA may have to be determined for each sampling date. A major advantage is this method becomes apparent when leaf and stem phytomass data are required in addition to leaf area data. The incremental costs of determining the leaf area/mass ratio for a subsample of leaves is small compared with the costs of determining phytomass (Daughtry and Hollinger, 1984). Thus the overall objectives of the experiment must be considered before selecting a method of measuring leaf area.

#### E. Optical Planimetric Methods

Optical planimetric methods require special equipment, e.g., automatic planimeters or video image analyzers. Numerous instruments employing planimetric principles are described in the literature (see reviews by Marshall, 1968; Kvet and Marshall, 1971; Ross, 1981). Problems associated with ambient light conditions and leaf optical properties that plagued early instruments are minimized electronically in current commercially-available instruments.

The first type of optical planimeter is a scanning planimeter (e.g., LI-COR LI-3000 in Table 1) which uses an electronic method of rectangular approximation. Object width is scanned by sequentially pulsing light emitting diodes (LED) located in the upper section of the scanning head and sequentially reading photodiode detectors located in the lower section of the scanning head. When the light from one or more LED's is interrupted by a leaf, its width is measured. The area of the leaf is measured as the leaf is drawn through the scanning head. In its portable mode, the LI-3000 area meter can measure leaf area nondestructively. The scanning head can be combined with a transparent belt conveyer for measuring large numbers of detached leaves. The conveyer belt travels at a constant speed and moves the leaves through the scanning head.

Other scanning planimeters (e.g., LI-3100) use a fluorescent light source and a

TABLE 1  
 Technical data supplied by the manufacturers of scanning optical planimeters.

	LI-3000	LI-3100		CI-201	CI-251
		1.0	0.1		
Sample dimension					
max width, mm	127	254	75	115	215
min width, mm	1	1.5	0.5	0.1	0.1
max thickness, mm	8	25	25	$\infty$	10
max length, m	1	$\infty$	$\infty$	$\infty$	$\infty$
Resolution, mm <sup>2</sup>	1	1	0.1	0.1	0.01
Accuracy	2%*	1%*	0.5%†	1%	1%
Power	battery	AC	AC	battery	AC
Weight, Kg	2.7	—43—		0.7	5
Approximate cost (1989)	\$5,500	—\$6,600—		\$4,000	\$4,000

\* for areas > 50 cm<sup>2</sup>.

† for areas > 10 cm<sup>2</sup>.

*Notes:*

1. LI-COR LI-3000A portable leaf area meter also has an optional AC powered conveyer assembly.
2. LI-COR LI-31000 laboratory leaf area meter may be configured in either the 1.0 mm<sup>2</sup> or 0.1 mm resolution modes. (Address: LI-COR, Inc., P.O. Box 4425, Lincoln, NE 68504, USA Phone 402-467-3576 FAX 402-467-2819)
3. The Morgan CI-201 portable leaf area meter was introduced in the late Fall of 1989. An optional conveyer assembly is available. (Address: P.K. Morgan Instruments, Inc., 2 Dundee Park, Andover, MA 01810, USA Phone: 508-470-0473; FAX 508-474-0137)
4. The CI-251 conveyer image analyzer was introduced in 1990. (Address: CID, Inc. P.O. Box 9008, Moscow, ID 83843 USA: Phone 1-800-767-0119).

solid-state scanning camera to sense the areas of objects as they move through the instrument (Table 1). Adjustable press rollers flatten curved leaves and feed them between the transparent belts. Both the LI-COR area meters have an optional read-out console which provides additional data on leaf length, average width, and maximum width for single leaves or groups of leaves.

A light weight portable scanning planimeter was introduced in the Fall of 1989 by P.K. Morgan Instruments (Table 1). This one-piece instrument uses a bar code reader to encode length as the sensor moves along a leaf. Leaf width is measured by light reflected from the leaf to the detectors, thus this instrument is not limited by thickness of the leaf. Leaf area can be measured nondestructively with this instrument. The CI-251 conveyer image analyzer, introduced in 1990, has very high spatial resolution and can store and transfer images to a computer for additional analyses.

With conveyer systems, care must be exercised to prevent leaves from lodging in the instrument. If a leaf becomes lodged it blocks the light reaching the detectors and the instrument will continue to accumulate area which will result in an erroneously high leaf area. Dust, dirt, and plant residues may contaminate the conveyer belts and contribute to spurious readings. Considerable care must be exercised to clean and maintain the belts. Conversely, if leaves fold or overlap each other as they move through the area meter, the leaf areas measured will be lower than the actual leaf areas. These instruments also "flatten" leaves during the measurement process and thus underestimate the area of leaves with ripples. For

example, leaf area measured on an intact maize leaf may be 4–8% less than when the leaf is cut into pieces and the areas of the pieces measured (Norman and Campbell, 1989).

The precision of the scanning planimeters is excellent. The coefficients of variation for 30 repeated measurements of a calibration disk, a soybean leaflet and a maize leaf were 0.1, 0.2, and 0.3%, respectively using the LI-3100 area meter (Daughtry and Hollinger, 1984). These random errors of measurement associated with the area meter are very small compared with other sources of variation. In general, the scanning planimeters have greatly improved the accuracy and efficiency of measuring leaf areas.

The second type of optical planimeters is the video image analysis system. These instruments are used for a wide variety of applications from aerial surveys to microscopy and from digitizing maps to inspecting food and manufactured products. State-of-the-art image analysis systems are modular so as to support a variety of applications. The majority of these systems have been designed for analysis of remotely sensed data, for geographic information systems, and for automated inspection. One can select from a wide range of systems both in terms of capabilities and costs. The costs of video image analysis systems range from several thousand dollars to several hundred thousand dollars or more.

Recently several turn-key systems have become available that are targeted specifically for agricultural/ecological applications. One such system is the Decagon Ag Vision System which can provide areas, sizes, shapes, and numbers of leaves (Table 2). Systems designed specifically for leaf area measurements are listed in Table 2. The basic system for measuring leaf area consists of a video camera, a frame digitizer, a monitor, and a computer with appropriate

TABLE 2

Addresses and telephone numbers for several companies offering video image analysis systems (hardware and software) for measuring area of leaves.

Vendor*	Equipment †	Approximate Cost, (\$)
Decagon	Monochrome Ag Vision System (includes light box and camera stand)	4,000
	Pseudocolor Ag Vision System (includes computer, light box and camera stand)	7,300
	Delta-T Area Measurement System	3,300
Skye	Leaf Area and Analysis System	4,000

\*Addresses and telephone numbers of vendors.

Decagon Devices, Inc. P.O. Box 835 Pullman, WA 99163 USA	Phone: 509-332-2756 Telex: 9102400036 DECAGON DEVICE UQ
Skye Instruments Inc. P.O. Box 198 Perkasie, PA 18944 USA	Phone: 215-453-9484 Telex: 517578

† Each system minimally includes a camera, a digitizing card, and appropriate image analysis software. A microcomputer must be supplied either by the vendor or the user. Each vendor offers many accessories to customize the equipment to the user's application.

software to analyze the digital data. Turn-key systems for determining leaf area typically have a standard video camera mounted on a copy stand and leaves are placed in front of the camera on a light box or a conveyer. An image of the leaves is digitized, enhanced, and analyzed to discriminate the leaves from the background. A high resolution color monitor is helpful for distinguishing subtle differences in leaf color or shape associated with some diseases and nutrient deficiencies. The computer calculates leaf area and a variety of additional parameters including the length, width, shape, and number of leaves in a scene. Statistical analysis including totals, means, and standard deviations are possible in most software packages.

The resolution of an image analysis system ranges from microscopic to macroscopic depending on the video camera and the optics used. Specialized software packages are available for determining leaf area, root length, number of objects, cell size and shape, as well as diseased or chlorotic areas on leaves. The spatial resolution of a video system is determined by the number of pixels (i.e., picture elements) in the detector array of the video camera and the optics used. A video camera will typically resolve approximately  $500 \times 500$  pixels (i.e., picture elements) while a high resolution camera may resolve  $1000 \times 1000$  pixels or more.

A video image analysis system may be used to determine the projected areas of small stems or conifer needles. Total surface areas of the stems or needles may be calculated based on their shape. Discrimination and quantization of diseased, chlorotic, and necrotic areas on leaves are also possible with video image analysis systems. Inoue (1989) discussed many of the principles of video enhancement and image processing in light microscopy that are broadly applicable. He points out that as the potential uses and economic practicality of video image analysis become appreciated, many other applications will be found in the basic and applied sciences.

### III. SURFACE AREA OF CONIFER NEEDLES, STEMS, AND OTHER PLANT ORGANS

The surfaces of conifer needles, stems, and other plant organs are complex and the areas are usually estimated by assuming a geometric shape and making linear measurements. For example, the stem surface of primary concern is not the microtopography of bark flakes and furrows but the imaginary smooth surface of rotation connecting the ridges. Thus the surface area of a stem may be calculated as that of a cylinder by taking its length and mean diameter. For a more accurate estimate of stem surface area, one may approximate the stem by other geometric shapes, such as a paraboloid or cone (Causton, 1985).

For a more complex problem, Shelton and Switzer (1984) estimated the surface area of loblolly pine needles by assuming that each fascicle could be represented by a circular outline with six internal faces (loblolly pine typically has 3 needles per fascicle). They measured length and diameter in several places along the length of the bundle of needles. Fascicle mass was also a good predictor of surface area although fascicle age and its position in the canopy affected the relationship.

The volume of needles (or other plant parts) may be measured by employing the Archimedes principle and calculating surface area for a particular geometric shape

based on the volume of water displaced. For example, Johnson (1984) estimated the total surface area of pine needles using (i) measurements of the displaced volume of the needle sample, (ii) the cumulative needle length of the sample, and (iii) the number of needles per fascicle. One advantage of this technique is that the diameter of individual needles is not required, only needle length is needed. For short-needled conifers, such as spruce and fir, Johnson (1984) proposed determining needle volume in a two step process. First, the volume of the intact branch with needles attached to the stem is measured and then the volume of the stem without the needles is measured. Needle volume is determined by subtraction. The accuracy of the volume displacement method to estimate surface area largely depends on the ability of the investigator to determine the appropriate geometric shape and to measure the required parameters for representative needles.

The surface area of needles can also be calculated by measuring the mass increase after covering the needles with an adhesive and a monolayer of small (0.08–0.11 mm) glass beads (Thompson and Leyton, 1971). Considerable care is required to ensure that the needles receive a truly uniform coating of beads and the method is subject to considerable "operator" technique. Davies and Benecke (1980) demonstrated that a fluidized bed of glass beads could greatly speed up the procedure and enhance uniform distribution of glass beads. A critical feature of the glass bead technique is the determination of a mass/area calibration factor suitable for the foliage under consideration. Various sizes of paper squares and gauges of wire have been used for calibrations depending on the characteristics of leaves or needles to be measured. For small complex surfaces, the glass bead technique could be used to calibrate leaf (or stem) surface area to leaf (or stem) phytomass relationships. The major disadvantages of the glass bead technique are that it requires a significant amount of time for a measurement and it is most suitable for a small number of needles.

The projected area of needles and small stems can be measured with an optical planimeter and total surface area can then be estimated using the shape of their cross-sectional areas. The scanning planimeters (e.g., LI-COR models) are well suited for stems greater than 2 mm but are only marginally suited for small conifer needles whose width (or thickness) may be less than 1 mm or approximately the spatial resolution of the instrument (Table 1). The CI-251 conveyer image analyzer (Table 1) should be capable of accurately measuring the projected areas of most conifer needles. With the proper optics, video image analysis systems can easily measure the projected area of small stems and conifer needles. Thus optical planimetry offers one of the best compromise solutions for measuring the surface area of needles provided needles lie flat and proper curvature correction factors are applied.

#### IV. PHYTOMASS OF LEAVES, STEMS, AND OTHER ORGANS

Direct measurement of phytomass involves harvesting and weighing plants. Masses may be determined on fresh or dried plants, although dry masses are generally more reliable. Water loss occurs rapidly when plants are harvested and accurate measurements of fresh mass are difficult to obtain. In some cases, it may

be advantageous to harvest and determine the fresh mass of a large volume of plant material and then take small subsamples to determine the proportion of dry matter. The dry mass of the whole sample can be calculated from the total fresh mass and the proportion of dry matter in the subsamples. This technique is useful for large species, e.g., trees or shrubs, or when oven space for drying samples is limited. A critical element in this technique is obtaining representative subsamples to minimize errors. A forage chopper or brush shredder may be used to chop and mix plant parts prior to subsampling. Alternatively one could subsample by plant parts and determine the proportion of dry matter in each plant part.

Plant samples should be dried at 60–80°C to constant mass rather than for a fixed time. High temperatures may cause loss of volatile compounds. Large samples take longer to dry than small samples. Thick stems may be split to speed drying.

Indirect measurements of phytomass are well-developed in forestry and the ecological sciences; they involve the use of an empirical relationship between a variable that is difficult to measure (total stem volume) and a more easily measured variable (diameter at breast height, dbh). When a mathematical relationship is fitted to data of the form  $V=f(D)$ , the regression of  $V$  on  $D$  assumes homoscedasticity (i.e., uniform variance) of  $V$  over the range of  $D$ . Frequently the variance of  $V$  (or other plant characteristics) increases as  $V$  itself increases and a logarithmic or another transformation of the quantities concerned is appropriate (Anderson and McLean, 1974; Causton, 1985). The general form of this relationship is:

$$\log V = \log a + b \log D \quad (3)$$

or the power form of the equation:

$$V = a D^b \quad (4)$$

where

$V$  = total stem volume (or other characteristic)

$D$  = stem diameter breast height, dbh

$a, b$  = regression coefficients.

Although the two equations are mathematically equivalent, they are not statistically equivalent. Different values of the constants,  $a$  and  $b$ , will be obtained if one uses least squares regression with the usual error structure of normal distribution of error with a zero mean (Causton, 1985). The expected value of  $\log V$  has a downward bias and various corrections and weighting schemes have been proposed (Anderson and McLean, 1974). Nevertheless the allometric variants of  $V = aD^b H^c$  give good estimates of stem volume from height ( $H$ ) and stem diameter ( $D$ ) measurements (Causton, 1985).

Other major components of the crown, which also may be estimated with simple allometry, include foliage area, foliage mass, and branchwood mass. The underlying concept is that a given amount of xylem or sapwood can physiologically and mechanically support only a certain amount of foliage. Thus foliage area and mass can be estimated if one measures the cross-sectional area of the sapwood (Long

*et al.*, 1981). Bole (stem) diameter at the base of the crown is also frequently cited as the best single estimator of foliage and branchwood weights, but diameter at breast height (dbh) and crown ratio (crown length/total stem length) used together are satisfactory. Although these equations must be calibrated for each species, there seems to be some consistency in the coefficients. Numerous reviews of these techniques are available including Causton (1985) and Whittaker and Marks (1975).

## V. FOLIAGE CONFIGURATION

The radiation scattering properties of a plant canopy depend not only on the quantity of foliage present but also on the location, inclination, and orientation of the foliage elements. The configuration of foliage that is most efficient in intercepting and utilizing incoming radiation seems to vary with species and environments.

Fundamentally six variables ( $x$ ,  $y$ ,  $z$ ,  $\Theta$ ,  $\phi$  and  $t$ ) are required to describe the location ( $x$ ,  $y$ ,  $z$ ) and direction ( $\Theta$ ,  $\phi$ ) with time ( $t$ ) of each piece of foliage in the canopy. The minimum set of geometric data required by current optical reflectance models is height ( $z$ ) and angle from vertical ( $\Theta$ ) of foliage. At least two additional variables ( $x$  and  $y$ ) must be included to model the radiation scattering by vegetation in row crops.

Foliage distribution within a plant canopy may be determined by stratified sampling, i.e., the harvest of successive layers of foliage (Ross, 1981). Leaf area, leaf inclination, leaf and stem phytomass, and other desired characteristics are determined for each layer. The thickness of each layer depends on the objectives of the study and the height of the plants. Generally this method is suitable for relatively short stands, i.e., less than 3 meters tall. Taller stands are difficult to stratify, harvest, and weigh. Stratified sampling can quickly become very time consuming and tedious if the canopy is divided into many layers and plant parts.

Leaf angle is generally defined as the angle between the normal (perpendicular) to the leaf surface and the horizontal plane or vertical direction. Leaf orientation is the azimuth of the horizontal projection of the leaf axis, usually measured clockwise from North. In practice some plants pose special problems for these definitions of foliage angle and orientation. For example, the long, thin leaves of many grasses curve downward near the tip. Leaf angles must be measured for each segment of each leaf. Some grass leaves (e.g., maize) also have wavy margins which increases the complexity of the leaf surfaces. In other plants, the leaves (or petioles) may twist and the abaxial surface of the leaves may face upward.

The most reliable direct method of measuring foliage angle and orientation uses a protractor, a compass, and a ruler (Ross, 1981). The angle of each leaf or leaf segment is measured with a protractor. A compass is used for azimuthal orientation and the ruler is used for distances from some reference point (i.e. soil surface or an arbitrarily defined plane). This method is most suitable for relatively short broadleaf plants, but can be used for segments of grass leaves.

Many leaves must be measured to characterize the frequency distribution of

angles and orientations. To illustrate this point, if one divides a plant canopy into 10 layers, measures 5 leaf inclination angle classes, and assumes uniform leaf azimuthal orientation, there are 50 possible cells in the array. If one uses the rule of thumb for the minimum number of observations as 10 times the number of cells, then more than 500 randomly selected leaves must be measured to determine the frequency distribution of leaves in this canopy. Furthermore, since the objective of the leaf angle distribution measurement is to obtain the area distribution of leaf angles, many bits of leaf area must be measured to characterize a single leaf. Thus, the measurement of the angles and locations of many bits of leaf area or more than 500 leaves with these simple tools is a formidable task and practically impossible if the canopy structure changes with time, e.g., due to moisture stress, phototropism, and wind. Nevertheless this technique is the standard for comparing indirect techniques of measuring foliage angles.

Lang (1973) described an elegant computer-assisted device for measuring canopy architecture that greatly increased the speed of data acquisition. His device used precision potentiometers to record the angles of three arms used to measure the three Cartesian coordinates that define the position of any chosen point on a foliage element. By selecting an appropriate array of points on any given leaf the position, inclination, azimuth, and area of any triangle enclosed by these three points are measured directly. Lang (1990) provides additional information on his device and techniques in Chapter 5 of this volume.

Vanderbilt (1985) described conceptually an optical radar system that is potentially capable of providing the most fundamental type of structural data, i.e., the area and direction of the normal of each small piece of foliage in each cube of space in the canopy. The potentially overwhelming size of the data set needed to characterize the structure of a plant canopy can be reduced considerably by appropriate statistical methods.

Foliage angle distributions can be determined from photographs of individual plants taken from two or three suitably chosen directions (Smith *et al.*, 1977). The photographs are digitized by placing a transparent grid over the photograph and recording the two-dimensional coordinates of straight line segments along the plant profiles. Data from each photograph are interpolated for individual leaves and three-dimensional coordinates of foliage elements are determined. Foliage inclination angles and element height distributions are calculated. For many vegetation species it is necessary to project only two directions to determine foliage angles, but for some species three orthogonal (two in the horizontal plane and one the vertical direction) photographs are required.

The photographic method is suitable for moderate-sized plants, less than 3 m tall, with few leaves, e.g., maize. Acquisition of the photograph is rapid, but determining angles from the photograph is slow. If the plants must be removed from a canopy for taking the orthogonal photographs, then all information on leaf azimuthal orientation is lost. Alternatively the surrounding plants could be destroyed and the remaining "island" of undisturbed plants photographed and analyzed. Photographs are not suitable for plants with many small leaves because some leaves will be hidden in the photographs.

The inclined point quadrat was originally developed for determining ground

cover in grass stands. Warren Wilson (1963) substantiated and used the technique for determining the vertical distribution and the mean inclination angle of foliage in plant canopies. Anderson (1971) thoroughly reviewed the technique. Estimation of LAI using this technique requires assumptions and equations similar to those described by Welles (1990) in Chapter 3 of this volume.

The inclined point quadrat method consists of piercing a plant stand with a long thin needle (point quadrat) in a specified direction and zenith angle and counting number of contacts of the point quadrat with vegetation in the canopy. Each contact with vegetation is scored and a contact frequency is determined. The method is nondestructive and in theory allows for repeated measurements but in practice the surrounding area may be trampled. The point quadrat method also allows estimation of statistical characteristics of radiation intensity within the stand.

The principal disadvantages of the point quadrat method are that large numbers of insertions (typically at least 1000) are required for reliable results (Ross, 1981) and there is some ambiguity in interpreting geometrical structure of plant canopy from the data (Philip, 1965). Any movement of the foliage during the measurement process increases uncertainty of the measurements and thus the canopy must be protected from wind. This technique is most applicable to stands less than 1.5 m high due to physical length of needles required.

Several notable modifications of the inclined point quadrat method have been developed. First, Miller and Lin (1985) described a drop-line method for a maple forest in which a line with a plumb bob is dropped through a tree canopy and the number of contacts with foliage elements are scored. This method requires a ladder or an aerial lift truck (cherry picker) to allow access to all parts of the tree for scoring the contacts of the line with the foliage which can be very difficult in a dense canopy. Second, Vanderbilt *et al.* (1979) modified the point quadrat technique by using a laser instead of a needle as the point quadrat. They also proposed using a laser ranging device to automate data acquisition. Finally, Caldwell *et al.* (1983) designed a very clever automated contact detection system based on a fiber optics probe. This system employed an infrared light source channeled through fiber optics and a high resolution fiber optic reflective sensor as the point quadrat. When the probe nears a leaf, light from the source is scattered back to the detector and contact with a leaf is recorded automatically. Distance travelled by the probe into the canopy is also recorded automatically. The automatic contact detection by this system permits work in dense canopies and increases sampling speed considerably over that of conventional point quadrats.

## VI. ESTIMATING REQUIRED SAMPLE SIZE

If proper precautions are taken, many of the methods discussed earlier in this chapter give sufficiently accurate measurements of leaf area and phytomass of individual plants. However, the variability among plants is an additional source of experimental error that must be considered in order to estimate the LAI or phytomass per unit area of soil. Even in "uniform" crop canopies the inherent variability in leaf area and phytomass per plant may exceed 10% (Daughtry and

Hollinger, 1984). In natural stands the variability is much larger and sample size must be considered carefully.

A researcher needs to know the number of measurements of each canopy property that must be acquired to detect differences among plant canopies with the desired confidence. If the researcher does not acquire enough samples per field (or experimental unit), his estimates of true LAI (or other plant characteristic) of a field will be too inaccurate to be useful. Conversely, the researcher also wants to avoid taking more measurements per field than are required because that limits the scope of the experiment.

Until recently most applied statistical work focused on minimizing the probability of Type I errors ( $\alpha$ ) and largely ignored the probability of Type II errors ( $\beta$ ). Type I errors essentially deal with the problem of finding a difference that does not truly exist, while Type II errors deal with failing to find a difference that truly exists (Cochran, 1963). When the cost of an experiment is considered, a prudent researcher must assess the probability of successfully detecting the effect he is looking for, even if such an effect does exist in the population. One measure of the probability of correctly rejecting a false null hypothesis ( $H_0$ ) is "power" which is  $1 - \beta$ , or 1 minus the probability of failing to find a difference that really exists (Howell, 1987). A more powerful experiment has a better chance of rejecting a false  $H_0$  than does a less powerful experiment.

The power of an experiment is a function of (i) the probability of a Type I error ( $\alpha$ ), (ii) the true alternative hypothesis ( $H_1$ ), and (iii) the sample size. A thorough development of the concept of the power of an experiment is found in Cohen (1969). Howell (1987) ably describes a good approximation of the true power of a test (Anderson and McLean, 1974; Cochran, 1963) and points out that researchers care not whether power = 0.85 or 0.83, but rather whether power is in the 0.80's or 0.30's. A careful statistical analysis beforehand will help a researcher in effectively designing the experiment.

Daughtry and Hollinger (1984) calculated that a minimum of 21 corn plants from a "uniform" field (i.e., CV = 10% for leaf area per plant) was required to detect true differences in leaf area of 10% at  $\alpha = 0.05$  and  $\beta = 0.1$  (power = 0.9). Their data showed that if one measured only 5 plants, the probability of successfully detecting 10% true differences would be 0.3 (Table 3). This means that if the

TABLE 3

The minimum number of plants required to detect differences among treatments as a function of the probability of success (power) and the true difference/CV ratio (gamma) using  $\alpha$  0.05 test of significance. In the example discussed in the text, the CV is 10% of the mean. Thus the gammas in this table correspond to true differences among treatments ranging from 5 to 50%.

Power	Gamma = true difference/CV					
	0.5	1.0	1.5	2.0	2.5	5.0
	..... number of plants .....					
0.9	85	21	10	5	4	1
0.7	50	13	6	4	2	1
0.5	30	8	4	2	2	1
0.3	17	5	2	2	1	1

null hypothesis of no significant differences among treatments is false and a 10% difference in LAI truly exists, it would be detected only 30% of the time. This is rather discouraging, since it also means that 70% of the time the researcher would make a Type II error.

What are the researcher's options? He could set  $\alpha$  at 0.1, thus increasing power to nearly 0.5, but this may not be acceptable. Other alternatives are to increase sample size or to adjust  $H_1$  by increasing the minimum acceptable difference between  $H_0$  and  $H_1$ . Table 3 illustrates the effects of the ratio of the true treatment difference divided by the coefficient of variation ( $\gamma$ ) on the number of samples required for various powers. If one wants to detect a 10% true difference when the CV is 10% ( $\gamma = 1.0$ ), a sample size of 8 plants gives a 50% chance of detecting the difference (power = 0.5). At least 21 plants are required for a 90% probability. On the other hand, if the researcher is willing to accept detecting only larger differences, e.g., 20% true differences ( $\gamma = 2.0$ ), then only 5 plants are required for a 90% probability (power = 0.9). These data also illustrate the value of reducing the standard error per unit or CV. One cannot have a high probability of detecting a significant difference with any reasonable number of replicates unless  $\gamma$  (true difference/CV) is greater than 1.0 (Table 3). Differences at least twice as large as the CV can be detected in most cases without excessive replication.

A first step for any researcher is to decide how small a difference among treatments must be detected, or conversely how large an error in leaf area (or other plant characteristic) can be tolerated. This demands careful thinking about the consequences of a sizeable measurement error. Initial estimates of sample size can be statistically evaluated and refined as experience is gained.

## VII. SUMMARY

The methods of measuring canopy structure vary greatly in their accuracy and ease of measurement. The natural variability in leaf area and phytomass per plant in "uniform" fields frequently exceeds 10% of the mean. Additional variability is introduced by methods which estimate leaf area based on area to mass ratios or measurements of leaf length and width. Optical planimetric measurements of leaf area have lower CV and require the fewer plants than the leaf area/leaf mass ratio method to detect comparable differences. Methods of estimating leaf area based on measurements of length and width of leaves require that more plants be measured but may be less time-consuming than the optical planimetric methods. These length and width measurement methods may be biased and the area coefficients must be verified frequently.

Six variables ( $x$ ,  $y$ ,  $z$ ,  $\theta$ ,  $\phi$  and  $t$ ) are required to describe the location and direction with time of foliage in a canopy. Direct measurements of the geometric characteristics of plant canopies with simple measuring tools are not adequate to describe canopies that change with time. This lack of accurate and extensive geometric data has delayed the development and testing of physically-based

models of radiation scattering in canopies. A capability is needed to determine this geometric information at the most fundamental level.

Finally when the true differences among treatments exceed 3 times the CV, most methods of measuring canopy structure 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 phytomass is also desired. Efficient and creative multistage sampling schemes can minimize experimental errors and costs. A preliminary sampling, followed by a statistical analysis, is very helpful in designing experiments for collecting the structural data of plant canopies.

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