GEOMETRY OF TOBACCO LEAVES: EFFECT ON ESTIMATION OF LEAF AREA[1]

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Tobacco plants of the broad and very broad genotypes were grown in the Phytotron to determine the effects of genotype, temperature during growth, age, and stalk position on rectilinear characterization of leaf shape. The two aspects of leaf shape that were evaluated were: intercept ratio (I), the ratio of total length of the midvein to the distance along the midvein from the base of the petiole to the intercept of maximum width; and relative base width (W_r), the maximum width of the leaf relative to the width across the base of the winged petiole. Variations in temperature-environment effected changes in I, while variations in genotype and stalk position were characterized by changes in W_r. Variations in the age of leaves was mutually characterized by changes in I and W_r.

The surface areas of individual tobacco leaves are frequently estimated according to the relationship $A = b_0LW$, where b is some proportionality constant relating area (A) to the product of length and width (LW). However, when a single, fixed b_0 was used to apply this relationship over a population of leaves of different ages and genotypes and from different stalk positions and environments, deviations were found between the actual and the estimated leaf area. These deviations were correlated with variations in I and W_r. Regression analysis was used to derive the equation

$$A = .6639[1 + .3803(1.31 - I^{1/3}) + .1784(2.19 - W_r^{1/2})]\ LW$$

in which the proportionality between A and LW is adjusted for changes in I and W_r. This equation appears to provide a more sensitive comparison among leaf areas within investigations that include multiple varieties, locations, plant ages, or stalk positions.

The geometry of tobacco leaves is an important criterion in genetic and production research. The shape of leaves is frequently used to characterize tobacco varieties (Humphrey, Matzinger, and Mann, 1965; Povilaitis, 1967), as well as to indicate environmental alteration of crop response (Raper, 1973). The surface area of tobacco leaves is of mutual concern to agronomists, as an indicator of yield potential; and ecologists, as a factor in photosynthetic potential. Many studies in these disciplines can benefit from a nondestructive analysis of leaf geometry, particularly those studies which include dynamic changes in plant growth.

Several systems have been proposed for conversion of rectilinear measurements of tobacco leaves to expressions of leaf surface area. For most of these systems, a linear correlation has been assumed between the product of leaf length and width (LW) and leaf area (Goff, 1895; Gubenko, 1939; McKee and Yocum, 1970; Suggs, Beeman, and Splinter, 1960; Tejawani *et al.*, 1957); in at least one (Splinter and Beeman, 1968) a correlation is assumed between stalk diameter and total leaf area of the plant. None of these methods, while they may be quite valid within their experimental constraints, have wide-range applicability and hence fail to account for a full varietal, age, or positional range of leaves with a single, simple equation.

While Suggs *et al.* (1969) state that leaf shape has no consistant effect on the correlation between LW and the leaf area, we propose that failure to qualify leaf shape is precisely what limits the applicability of the various published methods. There are three rectilinear characteristics of leaf shape that apply to tobacco: the ratio of length to width (L/W); the ratio of total length (L) of the midvein to the distance (L_i) along the midvein from the base of the petiole to the intercept of the axis of maximum width; and the maximum width (W) of the leaf relative to the width (W_b) across the base of the winged petiole. We have defined the latter two aspects of leaf shape as intercept ratio (I) and relative base width (W_r) by the equations

$$I = L/L_i \qquad \textit{1a}$$

and

$$W_r = W/W_b. \qquad \textit{1b}$$

As argued by Suggs *et al.* (1960), using in example an ellipse and its special case the circle, L/W of a geometric shape does not alter area; rather area is dependent only upon LW. However, both I and W_r can affect the area of curvilinear geometric shapes analogous to tobacco leaves.

In example of dependency of area on I, consider the enclosed portion of strophoidal curve **(Fig. 1A)**, a shape which resembles a tobacco leaf and is defined by the formula

$$a^2 = b^2\,[(L-b)/(L+b)], \qquad \textit{2}$$

where L is the maximum length along the axis of abscissas (length-axis) and *a* and *b* are the ordinate and abscissa, respectively, for any point on the perimeter. If shape is distorted by the allometric relationship

$$L = cW^k, \qquad \textit{3}$$

where *c* is a proportionality constant and *k* is a ratio constant defining the relative change in length and width, the area (A) of the strophoidal outline can be shown by integration to approach

$$A_0 = b_0LW = b_0L'W' \qquad \textit{4}$$

where b_0 is a coefficient relating the product of length (L or L′) and width (W or W′) to area. Thus, as was

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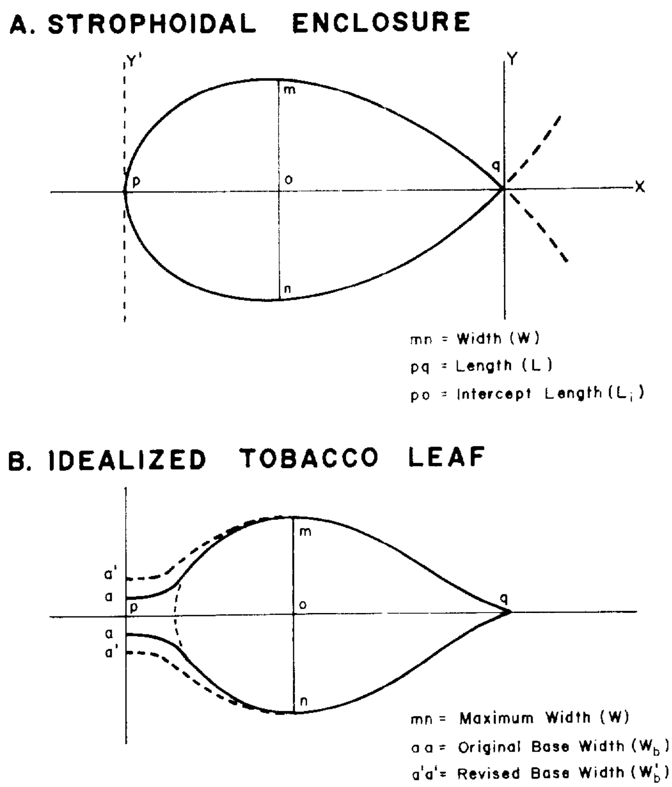

Figure 1. Dimensioned plots of enclosed portion of strophoidal curve (A) and idealized tobacco leaf (B).

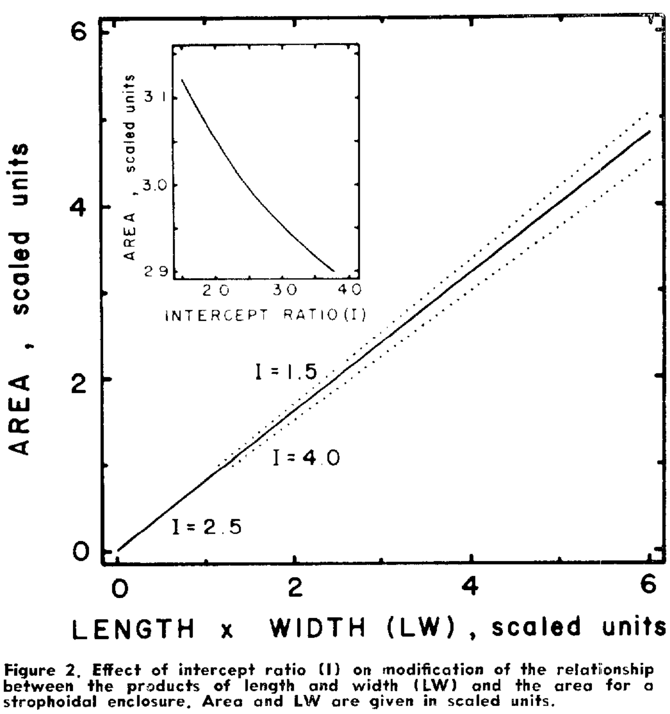

Figure 2. Effect of intercept ratio (I) on modification of the relationship between the products of length and width (LW) and the area for a strophoidal enclosure. Area and LW are given in scaled units.

illustrated by Suggs *et al.* (1960), area is independent of changes in the ratio of length to width as long as the distortion is at a uniform rate along the length-axis and the intercept ratio is unchanged. But, if the distortion along the length-axis is at an accelerated relationship to changes in width, i.e.

$$L = c(W^k)^k, \quad 5$$

the area of the resulting deformed strophoidal outline is directly related to the variation in intercept ratio. This is illustrated in **Fig. 2.** The solid line shows the linear relationship of equation [4] between the area and the product of length and width; the superior and inferior dotted lines show the relationship when the intercept ratio is distorted from the definitional I = 2.50 of equation [2] to I = 1.50 and I = 4.00, respectively.

To illustrate the dependency of area on W_r, we have constructed the idealized tobacco leaf in **Fig. 1B.** We replotted this idealized leaf for changes in L and W while maintaining constant values of W_r and I. Then, for each of these LW conformations, we replotted the perimeters for a succession of values for W_b, by distributing the change in W_b along L_i according to the imposed relationship

$$a' = W - [(W-a)\ (W-W_b')/(W-W_b)], \quad 6$$

where *a* and *a′* are original and revised widths at any point along the length axis between the base of the petiole and the axis of maximum width and where W_b and W_b' are the original and revised base widths. As shown in **Fig. 3**, the areas of the resulting deformations of a simulated leaf outline, determined photometrically (c.f. "Methods"), are related to variations in W_r. The solid plot shows the relationship of equation [4] for the original W_r; the superior and inferior dotted lines show the revised relationships when W_r is altered from the original W_r = 4.30 of **Fig. 1B** or W_r = 2.00 and W_r = 10.00 respectively.

The shape of tobacco leaves, as measured by length to width ratio, is affected by an interaction between four distinct genotypes (Humphrey *et al.*, 1965) and the ambient temperature during growth (Raper, Johnson, and Downs, 1971; Raper and Thomas, 1972). Limited observations suggest that this interaction also affects I and W_r. The objectives of this experiment, therefore, are (1) to examine the respective contribution of genotype and temperature on alteration of I and W_r of tobacco leaves, and (2) to evaluate the effectiveness of these two rectilinear characteristics of leaf shape for predicting the variations in the relationship between area and LW of tobacco leaves which occur among varieties, environments, age, and stalk position.

METHODS

Tobacco seed representing the four genotypes for leaf shape superimposed on a common genetical background were obtained from D. G. Matzinger (Department of Genetics, N. C. State University). These seeds were sown in individual 250 cm^3 plastic pots filled with a peat-vermiculite substrate. Seed of the genotypes representing the narrowest (PtPtPdPd) and medium narrow (PtPtpdpd) failed to produce viable seedlings. Seedlings representing broad (ptptPdPd) and very broad (ptptpdpd) genotypes (exemplary of 'Coker 139' and 'Dixie Bright 244' varieties) were grown to transplant size in the 26/22 C (day/night temperatures) greenhouse unit of the N. C. State University Phytotron. Six plants of each genotype were transplanted into 25.4-cm diameter plastic pots filled with sand. Three of the six plants of each genotype were placed in a controlled-environment room (CER) with a 26/22 C temperature regime; the remaining three plants, in a CER with a 18/14 C temperature regime. Both CERs had a light regime of 9 hrs of high intensity fluorescent and incandescent light (450 hectolux) during the day period and a 3-hr interruption of low intensity incandescent light (32 hectolux) during the dark period. All cultural conditions were as described by Raper *et al.* (1971).

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Leaves from one plant of each genotype-temperature combination were removed two weeks after transplanting, identified, positioned on an easel, and photographed with 35 mm color reversal film (2x2 slides). The second plants of each genotype-temperature combination were continued in the respective CERs until the fourth week after topping. The third plants of each combination were shifted to the 26/22 C greenhouse after three weeks in the CERs and continued there until the fourth week after topping. Since the initial post-transplant period is critical in determination of the length/width ratio of mature, upper leaves (Raper and Thomas, 1972), these plants should provide an indication of whether or not this initial post-transplant period is also effective in determination of the intercept ratio component of leaf shape and size. All leaves from these mature second and third plants of each original genotype-temperature combination were removed at the end of the fourth week after topping, identified, positioned on an easel, and photographed.

The slides of each leaf were projected to true scale on opaque paper. The outlines and midveins of the leaves were traced from the projections and the tracings cut out. The actual surface area of tracings were measured photometrically with an Automatic Area Meter (Type AAM-5, Hayashi Denko Co., Ltd., Japan).[3] Length along the midvein (L), maximum width (W), width across the base of the winged petiole (W_b), and length from the base of the petiole to the intercept of the axis of maximum width and the midvein (L_i) were measured to the nearest 0.5 cm. Intercept ratio (I) and relative base width (W_r) were calculated by equations [1a] and [1b].

We feel that the technique of tracing the outlines and midveins of leaves from projected slides yielded accuracies of linear measurements equal to or greater than would have been obtained from measurements taken directly from the fresh leaves. In fact, for a small subsample of the photographed leaves, the linear measurements taken from tracings exactly corresponded with those taken directly from the fresh leaves prior to photographing.

RESULTS AND DISCUSSION

The contribution of genotype, temperature environment, leaf age, and leaf-stalk position to variations of intercept ratio (I) and relative base width (W_r) are summarized in **Table 1**. In general, we found little or no contribution of genotype or leaf-stalk position to fluctuations in I but recorded pronounced effects of the temperature environment during growth and of the age of leaves on I. These latter two treatment variables dominated the various interactive effects. In example, a comparison of genotypic response between 26/22 and 18/14 C temperatures shows that the lower temperatures emphasized the slight, but nonsignificant, tendency for lesser I values of leaves of the very broad genotype. Conversely, we recorded a dominant contribution of both genotype and age of leaves, but indistinctive effects of temperature, to fluctuations in W_r. The leaves did have a tendency for decreasing values for W_r towards the top of the plant. From these results, we expect varietal and positional variations in the coefficient (b_o) relating LW to area (equation [4]) to be associated with changes in W_r; while environmental variations in b_o, to be associated with changes in I. Variations in b_o due to age of the leaves from seedling to maturity would appear to be an interaction between changes in both I and W_r.

The effective period of temperature on the length to width (L/W) component of leaf shape is primarily confined to early stages in leaf development and quite probably to the stage of cell division (Raper and Thomas, 1972). Our present data indicate that the effective period of temperature on the I component of leaf shape is confined to later stages of leaf growth (i.e., cell expansion stages). Consider the I for leaves of plants grown for the initial three weeks after transplanting in either 26/22 or 18/14 C temperatures and then transferred for the remainder of growth to a common, 26/22 C greenhouse environment (26/22-26/22 C and 18/14-26/22 C conditions). There was only a slight, nonsignificant effect of the initial temperature conditions on I.

To evaluate the ability to predict changes in the coefficient (b_o) relating LW and area of tobacco leaves from variations in I and W_r, we first had to obtain parameters which satisfactorily define the relationships between b_o and I and W_r. The inserts in **Figs. 2** and **3** depict variations in area of leaf-like shapes which occur with variations in I or W_r, respectively, when all other rectilinear characteristics are maintained constant. Area is related to independent changes in I or W_r by the power functions.

Table 1. One-tailed "t" tests for variations in intercept ratio (I) and relative base widths (W_r) of tobacco leaves among genotypes, temperatures during growth, ages and stalk positions.

		Intercept Ratio			Relative Base Width		
Variable	df	Mean	Value of "t"	Probability of >t value	Mean	Value of "t"	Probability of >t value
Genotype:							
Broad	158	2.23	0.584	>.25	6.03	10.910	.001
Very Broad	158	2.20	0.584	>.25	3.56	10.910	.001
Temperature:							
26/22 C	97	2.13	−5.163	.001	5.12	0.506	>.25
18/14 C	97	2.41	−5.163	.001	4.92	0.506	>.25
ᵃ18/14 C	57	2.56	8.032	.001	4.29	−1.628	.10
18/14-26/22 C	57	2.12	8.032	.001	4.97	−1.628	.10
ᵃ26/22 C	70	2.21	0.955	.20	4.43	0.425	>.25
26/22-26/22 C	70	2.17	0.955	.20	4.29	0.425	>.25
26/22-26/22 C	59	2.17	1.181	.15	4.29	−1.563	.10
18/14-26/22 C	59	2.12	1.181	.15	4.97	−1.563	.10
Leaf Age:							
14 days	56	1.98	−4.691	.001	6.60	3.667	.001
Mature	56	2.24	−4.691	.001	4.70	3.667	.001
Stalk Position:							
Leaves 1-6	94	2.23	−0.887	.20	4.61	−0.203	>.25
Leaves 7-12	94	2.28	−0.887	.20	4.67	−0.203	>.25
Leaves 1-6	81	2.23	−1.539	.10	4.61	1.728	.05
Leaves 13-top	81	2.39	−1.539	.10	4.07	1.728	.05
Leaves 7-12	81	2.28	−0.587	>.25	4.67	1.722	.05
Leaves 13-top	81	2.39	−0.587	>.25	4.07	1.722	.05
Genotype within Temperature:							
18/14 C							
Broad	40	2.48	1.318	.10	6.02	4.494	.001
Very Broad	40	2.34	1.318	.10	3.70	4.494	.001
26/22 C							
Broad	55	2.15	0.540	>.25	6.19	5.649	.001
Very Broad	55	2.11	0.540	>.25	3.81	5.649	.001

ᵃ*Excludes leaves from 14-day old transplants.*

Table 2. One-tailed "t" test for deviation of various estimated b values from the actual b value.

Deviation from actual b	df	Mean of Deviations	Value of "t"	Probability of >t value
b^a — adjusted b^b		.0275		
b — b^c_o	158		−1.664	.05
		.0313		
b — adjusted b		.0275		
	158		−3.672	.005
b — Suggs' b^d		.0378		

ᵃ*b = actual area/LW.* ᵇ*adjusted b* $= b_o[1 + .3803(1.31 - I^{1/3}) + .1781(2.19 - W_r^{1/2})]$. ᶜ$b_o = .6659$. ᵈ*Suggs' b for flue-cured tobacco =* [illegible]

[3]*Trade names are given as a part of the exact experimental conditions and not as an endorsement to the exclusion of other products that may also be suitable.*

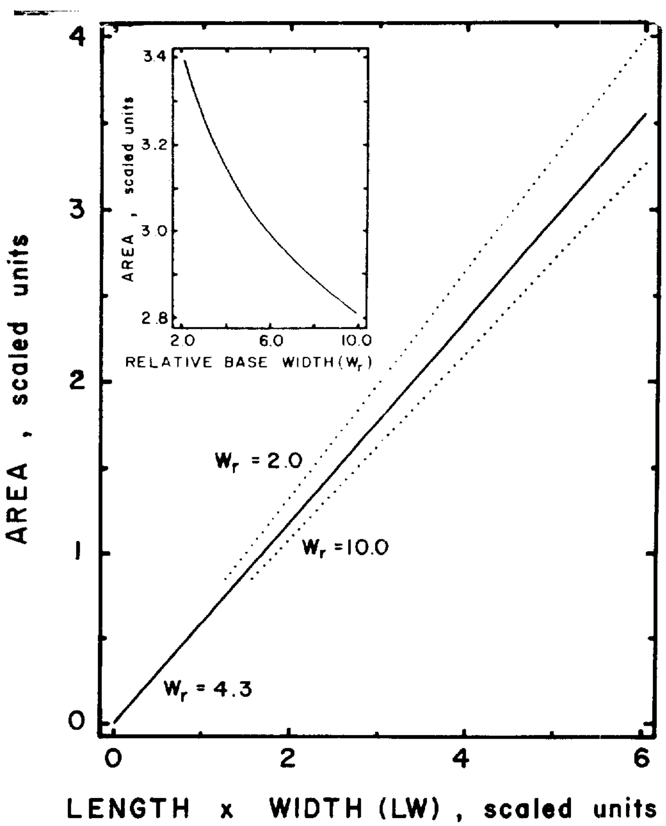

Figure 3. Effect of relative base width (W_r) on modification of the relationship between the products of length and width (LW) and the area for an idealized tobacco leaf. Area and LW are given in scaled units.

$$A = c_1 I^{k1} \qquad 7a$$

or

$$A = c_2 W_r^{-k2} \qquad 7b$$

where c_1 and c_2 are proportionality constants and k_1 and k_2 are ratio constants. The change in area with independent deviation of I or W_r from definitional values I_o and W_o can be adequately expressed by the relationships.

$$(A - A_{o1}) = c_1(I_o^{1/3} - I^{1/3})b_o LW \quad (r = .94) \qquad 8a$$

or

$$(A - A_{o2}) = c_2(W_o^{1/2} - W_r^{1/2})b_o LW \quad (r = .98) \qquad 8b$$

where A_{o1} is the area when $I = I_o$, A_{o2} is the area when $W_r = W_o$ and c_1 and c_2 are constants. If the independent deviations $(A - A_{o1})$ and $(A - A_{o2})$ are considered to be additive when I and W_r vary simultaneously, then

$$A - A_o = (A - A_{o1}) + (A - A_{o2}) \qquad 9a$$

where A_o is the area when $I = I_o$ and $W_r = W_o$. Furthermore, if A_o is defined by equation [4], then by substitution change in area with variation in both I and W_r can be expressed as

$$A - b_o LW = [c_1(I_o^{1/3} - I^{1/3}) + c_2(W_o^{1/2} - W^{1/2})]b_o LW. \qquad 9b$$

Multiple regression techniques were used to fit equation [9b] to our data characterizing variations in leaf area and shape among genotypes, temperature environments during growth, leaf-stalk positions, and leaf ages. Using the mean values of our data set for b_o, and I_o and W_o, we derived the equation

$$A - .6639(LW) = .6639(LW)\,[.3803(1.21 - I^{1/3}) + .1784(2.19 - W_r^{1/2})] \qquad 10$$

with the terms for both I and W_r providing significant contributions to the regression. The highly significant correlation for the regression ($r = .759$ with 316 df for the error term) indicates that much of the variation between actual area (A) and area predicted by the relationship of equation [4], typically attributed to errors in measurements of irregularities in leaf margins (Suggs *et al.*, 1960), can be explained by variations in I and W_r.

Equation [10] can be arranged as

$$A = .6639[1 + .3803(1.31 - I^{1/3}) + .1784(2.19 - W_r^{1/2})]LW \qquad 11$$

to predict the area of tobacco leaves by adjusting b_o for deviations in I and W_r. The range of actual b values ($b = A/LW$) within our data set was 0.5836 to 0.7408. As shown in **Table 2** the adjusted b significantly reduces the mean deviation from the actual b values ($b = A/LW$) when compared by a one-tailed "t" test to either the mean deviation of the b_o derived for our data set or the b of Suggs *et al.* (1960).

We consider that equation [11], with its adjustable b value, provides a better estimate of leaf area than equations which utilize a single, fixed b value. We further consider that estimation of leaf area by equation [11], rather than by equations with fixed b values, becomes of increasing importance when the investigation includes comparison of plant material from different locations with different environments, plant material encompassing the different genotypes for leaf shape, or plant material of different ages.

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