1991
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JA 195:
1991

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Reprinted from *Agronomy Journal*
Vol. 83, No. 6

JA 1957
1991

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ABSTRACT

Leaf area and specific leaf area (SLA) are important parameters in many agronomic and ecological processes, but can be difficult and expensive to measure. This study was made to test simplified methods of estimating pearl millet [*Pennisetum glaucum* (L.) R. Br.] leaf area and SLA. Leaf length, maximum width, area, and dry mass data were obtained at 2-wk intervals from plants grown in 75-L pots. Pots contained 85 kg of acidic, P-deficient Betis sand (sandy, siliceous, thermic Psammentic Paleustalf) and were treated with four P levels and two water treatments (stressed and nonstressed). Individual leaf area was estimated non-destructively with the following equations:

$$\text{Leaf area} = 0.68 \times (\text{leaf length} \times \text{maximum width}) - 0.114 \quad (R^2 = 0.955)$$

and

$$\text{Ln(leaf area)} = 2.08 \times \text{Ln(length)} - 3.53 \quad (R^2 = 0.939).$$

Individual leaf area and whole plant leaf area were calculated from leaf dry mass by the following linear and nonlinear equations:

$$\text{Leaf area} = 133.6 \times \text{Leaf mass} + 22.69 \quad (R^2 = 0.900),$$

and

$$\text{Leaf area} = 162.84 \times \text{Leaf mass}^{0.667} \quad (R^2 = 0.973).$$

Residual errors indicated that the nonlinear equation was more accurate for area estimation of small leaves ($\leq$0.20 g), and that leaf area data were heteroscedastic. Leaf dry mass was also used to calculate SLA by the nonlinear equation

$$\text{SLA} = 176.7 \times \text{Leaf mass}^{-0.216} \quad (R^2 = 0.918),$$

which gave excellent fit to experimental data independent of harvest date, P level and watering treatment. Our results demonstrate that pearl millet leaf area and SLA can be accurately estimated and easily simulated from simple regression equations.

LEAF AREA AND ITS RATIO to leaf dry mass, specific leaf area (SLA), are important parameters in many agronomic and ecological processes, including photosynthesis, transpiration, and field energy balance. Simple, accurate methods for estimating these parameters are therefore necessary for many applications, including crop simulation models. Although simple methods exist for estimating leaf area of some common crops, e.g., maize (*Zea mays* L.), sorghum (*Sorghum bicolor* Moench), and cotton (*Gossypium hirsutum* L.), we know of none reported for pearl millet. Furthermore, there are currently no well-known, simple methods of estimating crop SLA.

W.A. Payne, ICRISAT Sahelian Center, B.P. 12404, Niamey, Niger, West Africa; C.W. Wendt, Texas Agric. Exp. Stn., Rte. 3, Lubbock, TX 79401; L.R. Hossner, Dept. of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, and C.E. Gates, Dep. of Statistics, Brigham Young University, Provo, UT 84602. Received 13 Dec. 1990. *Corresponding author.

Published in Agron. J. 83:937-941 (1991).

Abbreviations: DAE, days after emergence; and SLA, specific leaf area.

Estimating leaf area from the equation

$$\text{Area} = C \times (\text{length} \times \text{maximum width}), \quad [1]$$

where C is an empirical coefficient, can provide nondestructive leaf area estimates to within 0.05 accuracy (Norman and Campbell, 1989). McKee (1964) used this approach to obtain a value for C of 0.73 for maize. Bonhomme et al. (1974) obtained values of 0.74 for sugarcane (*Saccharum officinarum* L.) and 0.64 for cowpea [*Vigna unguiculata* (L.) Walp.]. Stickler et al. (1961) reported a value of 0.75 for fully expanded sorghum leaves, while McCree et al. (1984) used a value of 0.68 for expanded and expanding leaves. We know of no such coefficient reported for pearl millet.

Leaf area can also be estimated for a number of plant species from a linear, log-log relation with leaf length (Wendt, 1967; Wendt et al., 1967). These authors stated that the log-log relationship between leaf area and leaf length had been found to exist in five species with widely differing leaf morphologies and may exist in all plant families. The obvious advantage of the log-log method is that it doesn't require leaf width measurements. No such equation has been reported for pearl millet.

Specific leaf area reflects leaf thickness and the relative proportions of assimilatory and conductive or mechanical tissues in leaves (Květ et al., 1971), and has been used to estimate crop leaf area (Rhoads and Bloodworth, 1964; Reddy et al., 1989) and leaf daily growth rate for partitioning of respiration (Kimura et al., 1978). The inverse of SLA, specific leaf mass, has been positively correlated with leaf water use efficiency among alfalfa (*Medicago sativa* L.) cultivars by Gutschick (1988), who reasoned that leaves with high specific leaf mass are cooler under a given radiation load due to higher stomatal conductance and lower water vapor pressure deficit. A decrease in water vapor pressure deficit increases water use efficiency (Tanner and Sinclair, 1983). Charles-Edwards (1982) has shown a positive correlation between SLA and light-use efficiency for several species. Thus, SLA is an important crop parameter to estimate.

The relations between SLA, growth stage, and environmental stress are not fully understood. As reviewed by Reddy et al. (1989), some scientists assume constant SLA for leaves after full expansion, while others maintain SLA varies with plant growth stage and the supply and demand for C. These authors showed that cotton SLA changes differentially with time and canopy layer, and can be correlated with mean daily flux of photosynthetically active radiation of the previous week. They hypothesized this was due to demand of growing parts for photosynthate, and to the resultant effect upon leaf starch content. Gibson (1975) observed an ontogenetic decrease of SLA in three field grown sorghum varieties. Similarly, McCree (1983) showed unambiguously that sorghum SLA decreased with increasing plant size under both controlled and field conditions.

Since larger plants may be expected to have larger leaves, it can be hypothesized that SLA varies with leaf mass. The objectives of this study were (i) to test two models that estimate pearl millet leaf area nondestructively, namely (a) the length-by-maximum-width method (e.g., Norman and Campbell, 1989), and (b) the log-log method used by Wendt (1967); (ii) to establish regression equations by which pearl millet leaf area can be calculated from leaf dry mass; and (iii) to test a statistical model that calculates SLA of individual leaves from their dry mass. It was a further objective that models be independent of plant age, nutrient stress, and water stress.

MATERIALS AND METHODS

Leaves for this experiment were harvested at 2-wk intervals from plants used in a growth analysis experiment conducted in the semiarid climate of Lubbock, Texas, during the summer of 1988 (Payne, 1990; Payne et al., 1991). Ten to twenty seeds of the pearl millet cultivar ICTP 8203 (Rai et al., 1990) were planted in 75-L pots lined with plastic and containing 85 kg of acidic, P-deficient Betis sand (sandy, silicious, thermic Psammentic Paleustalf). This soil was selected for its chemical, physical, and mineralogical properties, which were similar to those of sandy millet fields of Niger, Senegal, and Mali (Payne et al., 1991). The study consisted of a completely random experimental design with fixed effects due to P level and water treatment. Pots were treated with four P levels (0, 1.15, 3.38, and 7.77 g P m^{-2} in pots of 0.139 m^2 area). Each pot also received 128.1 g NH_4NO_3 m^{-2} and 40.3 g K_2SO_4 m^{-2}. Fertilizer was applied in powder form and thoroughly mixed into the upper 0.15 m of soil before planting. Phosphorus levels were subjected to two water treatments: water stressed and non-water stressed. The water stressed treatment was maintained at an average soil water content of 0.03 to 0.07 m^3 m^{-3}, whereas the non-water stressed treatment was maintained at an average soil-water content of 0.12 to 0.20 m^3 m^{-3}. Phosphorus levels and water treatments were randomly assigned to numbered pots.

At 14 d after emergence (DAE), plants were thinned to two plants per pot, and pot liners were sealed around plants to restrict water loss to transpiration. Average soil water content was determined with a load-cell balance calibrated in the field by adding known amounts of water to an empty pot. The amount of water required to maintain average soil water content within the specified range was determined twice weekly by weighing pots and calculating the average rate of transpiration for each watering level of each P rate. Additional experimental details are presented elsewhere (Payne 1990; Payne et al., 1991).

Five plants from each water treatment of each P level were randomly selected for harvest at 2-wk intervals after emergence, for a total of six harvests. At the first through fifth harvests, tops of plants selected for harvest were cut and immediately placed into large plastic bags with several moist paper towels. Bags were then quickly sealed and transported to a cool room so that leaves would retain turgor. At the first harvest, approximately 60 leaves were selected to obtain a wide range of lengths, but without regard to treatment; at the second through fifth harvests, two fully expanded, nondamaged leaves were selected from each plant, for a total of 10 leaves per treatment, or approximately 80 leaves per harvest. Within individual treatments, leaves were selected to obtain a wide range of lengths, and without regard to canopy layer.

Length and maximum width of selected leaves were measured to the nearest 1 mm. Leaf area was measured with a Li-Cor area meter (Li-Cor model LI-3100; Li-Cor, Inc., Lincoln, Nebraska[1]) which was calibrated with disks of known area. Measured leaves were placed in labeled paper bags and

[1] Mention of trademark names does not constitute an endorsement.

oven dried at 70 °C. After drying, leaf mass was determined to the nearest 1 mg.

For leaf area estimation, the MGLH module of SYSTAT (Wilkinson, 1987) (SYSTAT, Inc., Evanston, Ill.) was used to test three linear models. Model statements were

$$\text{(i) Area} = C \times (\text{length} \times \text{maximum width}) + \text{Constant} + E$$

for the length × maximum width method;

$$\text{(ii) Ln(area)} = M \times \text{Ln(length)} + \text{Constant} + E$$

for the log-log method; and

$$\text{(iii) Area} = B \times \text{mass} + \text{Constant} + E$$

to regress leaf area on leaf mass. In the above statements, C, M, and B represent empirical coefficients, and E is the error term. For obvious reasons, Models (i) and (ii) are only valid for leaf width and length >0.

The NONLIN module of SYSTAT (Wilkinson, 1987) was used to model leaf area and SLA as a function of leaf dry mass. The model statements were

$$\text{(iv) Area} = T \times \text{Leaf mass}^{Q} + E$$

and

$$\text{(v) SLA} = S \times \text{Leaf mass}^{P} + E.$$

Simplex minimization was used to estimate the parameters T, Q, S, and P, and required eight to 15 iterations to estimate their absolute magnitude to within a tolerance of 0.00005.

RESULTS AND DISCUSSION

Leaf Area

The relation between measured and calculated leaf area using length-by-maximum width [Model (i)] is shown in Fig. 1. The value of the coefficient C, 0.68, was lower than that reported for sorghum (0.75) (Stickler et al., 1961), sugarcane (0.74) (Bonhomme et al., 1974), and maize (0.73) (McKee, 1964), and equivalent to the value McCree et al. (1984) obtained for expanding and fully expanded sorghum leaves. Data indicate a high degree of association, and the low standard error of estimate for C (0.008) suggests that the relation accurately estimates pearl millet leaf area independent of age, water, and fertility. The model estimate of the additive constant, −0.114, did not differ significantly from zero.

A plot of Ln(leaf area) vs. Ln(leaf length) [Model (ii)] is shown in Fig. 2. The estimate of the coefficient M, 2.083, was similar to that found by Wendt (1967) for sorghum (2.152). The low standard error of the estimate of M (0.028) indicates that this model accurately estimated pearl millet leaf area; however, it slightly overestimated experimental values obtained at the low end of the curve.

Stickler et al. (1961) found that C varied only from 0.739 to 0.756 among six sorghum varieties studied; similarly, Wendt (1967) found minimal varietal change of M in sorghum. Therefore, although the values of C and M reported here ought to be verified before use with other pearl millet cultivars, they should be good approximations.

For non-destructive determination of leaf area of pearl millet, either of the above linear methods is appropriate. The length-by-maximum-width method appeared to be only slightly more accurate than the log-log method for this data set. Whether this slight gain in accuracy warrants measuring leaf width as well as leaf length would depend upon the nature of each investigation. However, our data suggest that for most applications, measuring leaf width to obtain leaf area may not be necessary.

Leaf area vs. leaf dry mass data (Fig. 3) used in

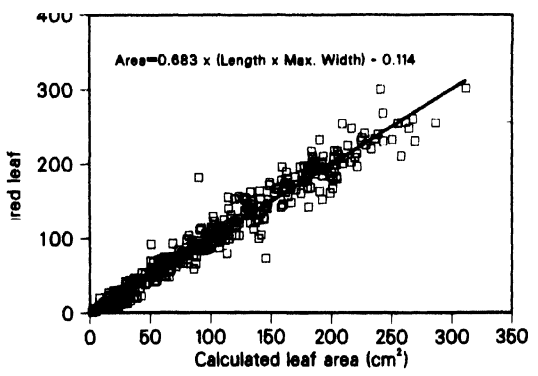

Fig. 1. Pearl millet leaf area calculated from length-by-maximum width vs. measured leaf area. Points are pooled across harvest, P level, and water treatment (n = 361, R^2 = 0.955, F ratio = 7556, and the std. error of coefficient C = 0.008).

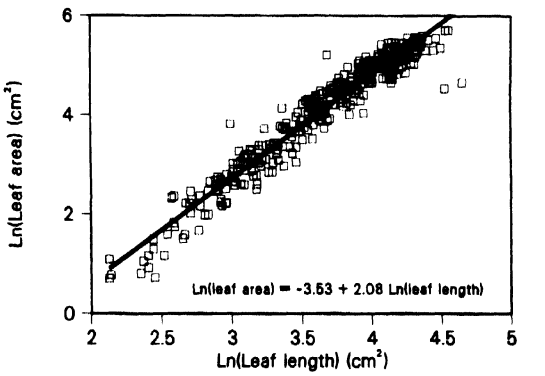

Fig. 2. Natural log of leaf area vs. natural log of leaf length for pearl millet. Points are pooled across harvest, P level, and water treatment (n = 361, R^2 = 0.939, F ratio = 5527, and std. error of coefficient C = 0.028).

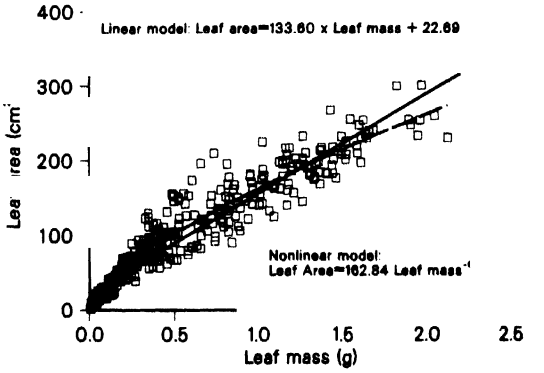

Fig. 3. Pearl millet leaf area vs. leaf dry mass. Points are pooled across harvest, P level, and water treatment (n = 361). For the linear model, R^2 is 0.900 and the std. error of slope is 2.34. For the nonlinear model, R^2 is 0.973, the std. error of coefficient T is 1.44 and the std. error of the exponential Q is 0.015.

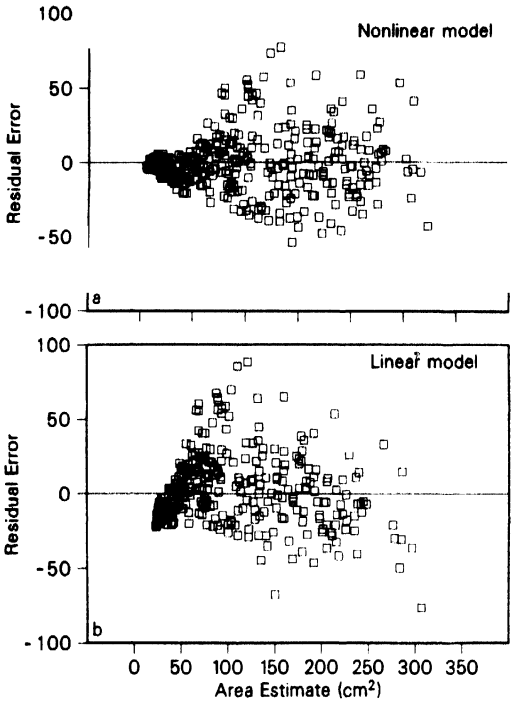

Fig. 4. Residual errors for leaf area estimation from leaf dry mass using (a) the nonlinear model and (b) the linear model.

Models (iii) and (iv) were more scattered than area vs. length-by-width (Fig. 1) and area vs. length (Fig. 2) data. Furthermore, as mass becomes ≤ 0.1 g, leaf area was overestimated by the linear model. The nonlinear model tended to be more accurate for small leaves. This fact is better illustrated by a plot of residual errors (Fig. 4). If Model (iii) had been forced through the origin; i.e., if the constant had been set equal to zero, then the model would have tended to overestimate leaf area. Thus, where leaves tend to be small due to growth stage or stress, the nonlinear model is more appropriate for leaf area estimation. The fan shape of residuals in Fig. 4a and 4b indicate heteroscedasticity in the leaf data, and so it may be argued that a weighted regression model is appropriate, with weights inversely proportional to observed variances. For example, the inverse square root of leaf mass could be used as a weight in the linear model to attenuate the fan shaped pattern of Fig. 4b.

To reiterate, the major advantage of using Models (iii) and (iv) is that weighing all leaves together without regard to their number is much less time consuming than measuring individual leaves with a ruler, as required by Models (i) and (ii), or measuring them one by one with an area meter. Obviously, neither destructive method is appropriate for small plant populations, e.g., growth chamber studies.

Specific Leaf Area

The slope of the plot of area vs. leaf mass (Fig. 3) represents an average SLA for this wide range of leaf mass. The fact that the linear model tends to underestimate leaf area of small leaves suggests that smaller

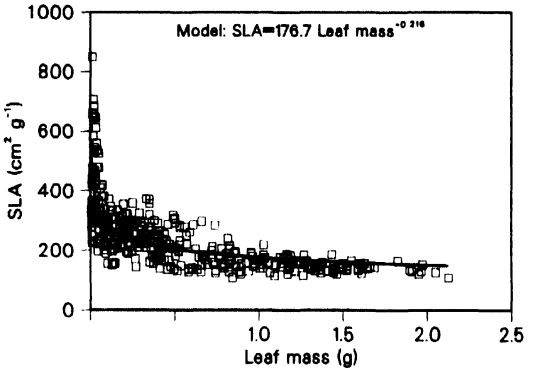

Fig. 5. Specific leaf area of pearl millet vs. dry leaf mass. Points are pooled across harvest, P level, and water treatment (n = 361, R^2 = 0.973, the std. error of the coefficient S = 4.7, and the std. error of the exponential P = 0.001).

leaves have more area per mass, or higher SLA. This nonlinearity may in part explain why dry matter production and leaf area are difficult to simulate during the seedling stage.

The plot of SLA vs. leaf mass (Fig. 5) confirms that small leaves tend to have greater SLA. A gradual increase in leaf SLA occurs as leaf mass decreases from 2.0 to 0.6 g, becoming greater from 0.6 to 0.1 g, and almost infinite at values ≤ 0.1 g. As data in Fig. 5 indicate, the greatest measured SLA was over 800 cm^2 g^{-1}. The model relating leaf mass to specific leaf mass gives an excellent fit to experimental data.

Leaf area for individual leaves can be calculated directly from area vs. mass relations, as shown in Fig. 3, or from multiplying mass by SLA. Relating SLA to leaf mass using an equation such as that shown in Fig. 5 renders the simulation of leaf area increase from dry matter increments much easier to simulate than trying to relate SLA to leaf canopy position and/or the flux density of photosynthetically active radiation (Reddy et al., 1989). Using equations of this type, one only needs to simulate the average dry mass of plant or canopy leaves, then calculate average SLA to obtain leaf area. Because an average SLA is required, one must determine the number of plant or canopy leaves when multiplying mass by SLA to estimate plant or canopy leaf area. However, when using Models (iii) or (iv), total area can be calculated directly from total mass without knowing leaf number. The choice of methods may depend upon leaf size. Our data set suggests that the range of SLA is narrow for leaves of large mass, but wide for those of small mass (Fig. 5). Conversely, the scatter of leaf area values for leaves of small mass is less than scatter for those of large mass (Fig. 3 and 4).

We have presented Models (iv) and (v) in nonlinear form simply because the data as shown in Fig. 3 and 5 appear to be nonlinear. A linear model could also be fitted to the data, i.e., Model (v) could readily be transformed to

$$\text{(vi) } \ln(\text{SLA}) = \ln(S) - P \times \ln(\text{leaf mass}).$$

However, in view of the extremely good fits that were achieved with nonlinear regression, this alternative was not explored.

It is appropriate to mention that values of the coefficients *C*, *M*, *B*, *T*, *Q*, *S* and *P* were found to be very stable independent of harvest (i.e., time), P level and watering treatment (data not shown). Since leaf-N concentration and plant-N uptake varied with P rate and watering level (Payne, 1990); leaf area and SLA can be calculated independent of time, nutrient stress, and water stress at least for this cultivar. The method should therefore be practical for many field and growth chamber applications. It should, however, be reiterated that even though leaves from stressed plants were used in this study, all models were developed using fully expanded, non-damaged leaves. Error is obviously introduced when attempting to use any of these equations for crops with large proportions of damaged or undeveloped leaves.

Finally, a disproportionate number of the small leaves used to develop the relationship in Fig. 3 and 5 were from water stressed and/or low P treatments, since these tended to grow very slowly. Therefore, they tended to have smaller mass and, as data in Fig. 5 indicate, higher SLA. Yet phosphorus stress increased leaf starch content in this millet cultivar (Payne, 1990). Therefore, in the case of phosphorus stress in pearl millet, it is not safe to assume that SLA is inversely proportional to starch content, as is normally expected.

ACKNOWLEDGMENTS

This study was partially funded by The United States Agency for International Development (AID) TropSoils CRSP Grant Dan-1311.

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