Prediction of Leaf Area in Individual Leaves of Cherrybark Oak Seedlings (*Quercus pagoda* Raf.)

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Abstract

The prediction of leaf area for cherrybark oak (*Quercus pagoda* Raf.) seedlings is important for studying the physiology of the species. Linear and polynomial models involving leaf length, width, fresh weight, dry weight, and internodal length were tested independently and collectively to predict leaf area. Twenty-nine cherrybark oak seedlings were grown in a greenhouse for one growing season and a total of 468 leaves were collected. Leaf area was polynomially related with leaf length or width, but linearly related with the cross product of length and width. Average leaf area for flush 3 was significantly greater than those of other flushes. However, variation in leaf area among flushes did not affect the models. Relationship between leaf area and length (or width) was consistent. Since leaf length is easy to measure and does not require destruction of leaves, it can be effectively used to predict leaf area in cherrybark oak seedlings.

Introduction

Leaf area is important in studying the physiology of trees. Most equipment to measure photosynthesis requires knowledge of leaf area to estimate photosynthetic rate and stomatal conductance. Although leaf size is usually large enough to fill leaf chambers of the equipment, leaf area must be estimated for smaller leaves. Similarly, to study the physiology of whole seedlings, leaf area for different sizes of leaves must be determined. Therefore, methods are needed to estimate leaf area quickly, accurately, and non-destructively.

Many methods to predict leaf area have been studied for various species. Although diameter, height, crown size, and root growth of trees have been related to leaf area (Bacon and Zedaker, 1986; Johnson et al., 1984), leaf length, width, and fresh or dry weight were most frequently used to predict individual leaf area (Farmer, 1980; Wargo, 1978; Persaud et al., 1993). Linear and polynomial models were generally used, applying the aforementioned factors as independent variables. For instance, Wargo (1978) related leaf length and width to leaf area and found that leaf area was closely related with the cross product of leaf length and width for black oak (*Quercus velutina* Lam.), white oak (*Quercus alba* L.), and sugar maple (*Acer saccharum* Marsh). Dry weight of leaves may also be used to estimate leaf area, but it may not be possible to preserve a foliage sample for drying. Therefore, fresh weight was suggested to replace dry weight of leaves (Larsen and Kershaw, 1991).

Another factor that may influence leaf area is the stage of seedling development. At stages of leaf expansion and flush lag, the interval between completion of one flush and the onset of the next, leaf morphology may vary (Hanson et al., 1986). Does the variation in leaf morphology affect the prediction of leaf area? Persaud et al., (1993) assumed that leaf blades of pearl millet (*Pennisetum glaucum*) have an invariant, genetically controlled shape and symmetry regardless of age and position on the plant. Can this assumption be applied to woody plant species?

Cherrybark oak (*Quercus pagoda* Raf.) is an important bottomland hardwood species in the southern United States, and research on the physiology of the species for successful natural regeneration has been conducted for many years (Hodges and Gardiner, 1993). One aspect of cherryback oak ecophysiology research is to study the influence of shade on photosynthesis and stomatal conductance of cherrybark oak seedlings, which requires developing a method to predict leaf area of the species. The objective of this study was, therefore, to apply leaf length, width, fresh and dry leaf weight, and internodal length in linear and polynomial models and to find the best models for predicting leaf area of cherrybark oak.

Materials and Methods

In 1989, half-sib acorns of cherrybark oak located on the Noxubee National Wildlife Refuge, Mississippi were collected and sowed 2 cm deep in PVC pots (35-cm in length and 15-cm in diameter). The post were filled with a 50:50 (v:v) sphagnum peat:sand mixture and placed in a greenhouse located at Mississippi State University. Potting medium was limed to pH 5.3. No artificial light was used. Twenty-nine seedlings were used for the experiment. The pots were irrigated 3-4 times per week with tapwater and once a week with a modified Hoagland's solution (Hanson et al., 1986; Hoagland and Arnon, 1939).

Once the first flush was completed, three seedlings were harvested. The same procedure was repeated for the next two flushes. A total of nine seedlings was harvested. Leaf area was measured by a Li-cor 3100 Leaf Area Meter. Length, width, and internode length for each leaf were measured to the nearest 0.1 cm. Length was measured from the tip of a blade to the connecting point of blade and petiole, and width was taken at the widest point. Leaf weight was recorded to the nearest 0.01 g and dry weight was measured after drying the leaves in an oven at 105° C for 48 hours. Upon termination of the study, the remaining 20 seedlings, all of which had completed at least three flushes of shoot growth, were harvested and measured similarly to the leaves mentioned above. A total of 468 leaves was used for the analysis. Data range and related statistics are listed in Table 1.

Table 1. Descriptive statistics of the 468 studied leaves.

Variable	Mean	Standard Deviation	Minimum	Maximum
Length (cm)	7.85	3.29	0.30	16.7
Width (cm)	4.55	1.88	0.20	10.0
Fresh Weight (g)	0.46	0.36	0.01	2.69
Dry Weight (g)	0.16	0.41	0.00	0.26
Internode Length (cm)	2.92	3.69	0.26	33.3

In order to study the relationship between relative leaf position on the seedlings and leaf area, we determined relative leaf position of each flush by computing the median of actual leaf position, and relative leaf position was determined accordingly. Internodal length was also compared to relative leaf position to determine if a relationship existed.

Data were analyzed using SAS (SAS Institute Inc., 1990). The data were fitted to linear and polynomial regression models. Leaf length, width, length x width, fresh weight, dry weight, and subtending internodal length were regressed with leaf area independently and collectively. The data from the nine sample seedlings had similar regression parameters to those of the remaining seedlings, and all the data were then pooled together. Analysis of variance was conducted to study the influence of flushes on leaf area. Duncan's Multiple Range test was used to separate means ($p=0.05$).

Results

Generally, leaf area was greater for the leaves in the middle of a flush, but only the bottom leaf and/or the top leaf were significantly smaller than others for flush 1 and 2. For flush 3 and 4, there was no significant difference among the leaves, although leaf area was greater for the leaves growing in the middle of the flushes (Fig. 1).

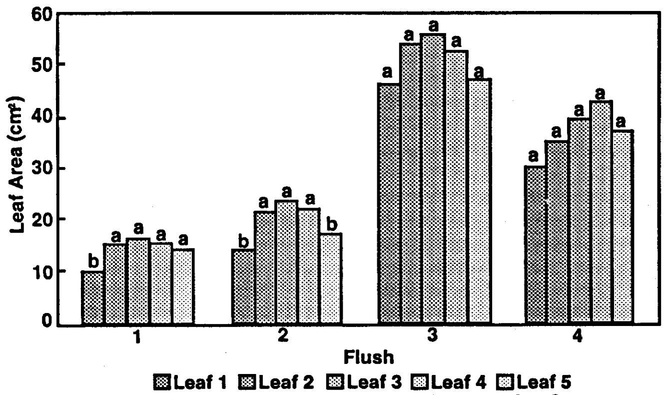

Fig. 1. Influence of relative leaf position on leaf area.

Average leaf area among flushes was significantly different (Fig. 2). Flush 3 had the largest leaves and flush 1 the smallest. The smaller leaf area for flush 4 compared to that of flush 3 was probably due to the final harvest before some of the leaves were fully expanded.

Despite the difference in leaf area among the flushes, the relationship between leaf area and leaf length was consistent for all the flushes (Fig. 3) since the overall relationship between leaf area and length was very close. Similarly, leaf width was also highly related to leaf area (Table 2). Both relationships were polynomial, with greater increases in leaf area at greater length or width.

The relationship between leaf area and the cross product of length and width was linear (Table 2). The regression coefficient improved slightly compared to that for length, with a r^2 of 0.98.

Fresh and dry leaf weight were linearly related to leaf area (Table 2), but the regression coefficients were relatively lower (0.79 and 0.86 respectively). Leaf area was not related to internodal length, although relative leaf position affected leaf area in flush 1 and flush 2. However, relative leaf position was closely related to internodal length, especially for flush 3 and 4 (Fig. 4).

Addition of fresh and dry weight to the models involving leaf length or width did not improve the fit. Multiple regression including leaf length, fresh weight, and dry weight produced a model with a negligible improvement in r^2. Similar results were produced for the model involving width, fresh weight, and dry weight.

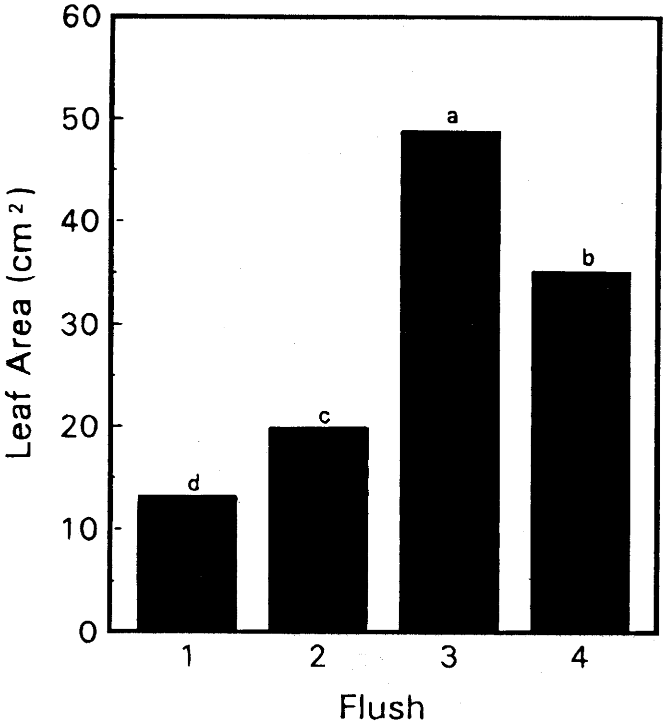

Fig. 2. Influence of flush on leaf area.

Table 2. Equations and related statistics for leaf width, length x width, fresh, and dry weight.

Variable	Equation	RMSE	r^2
Width	Leaf area = 2.56+1.00(Width^2)	4.73	0.94
Length x Width	Leaf area=1.45+0.610(Length x Width)	2.65	0.98
Fresh weight	Leaf area=4.22+48.98(Fresh weight)	8.81	0.79
Dry weight	Leaf area=1.53+191.5(Dry weight)	7.30	0.86

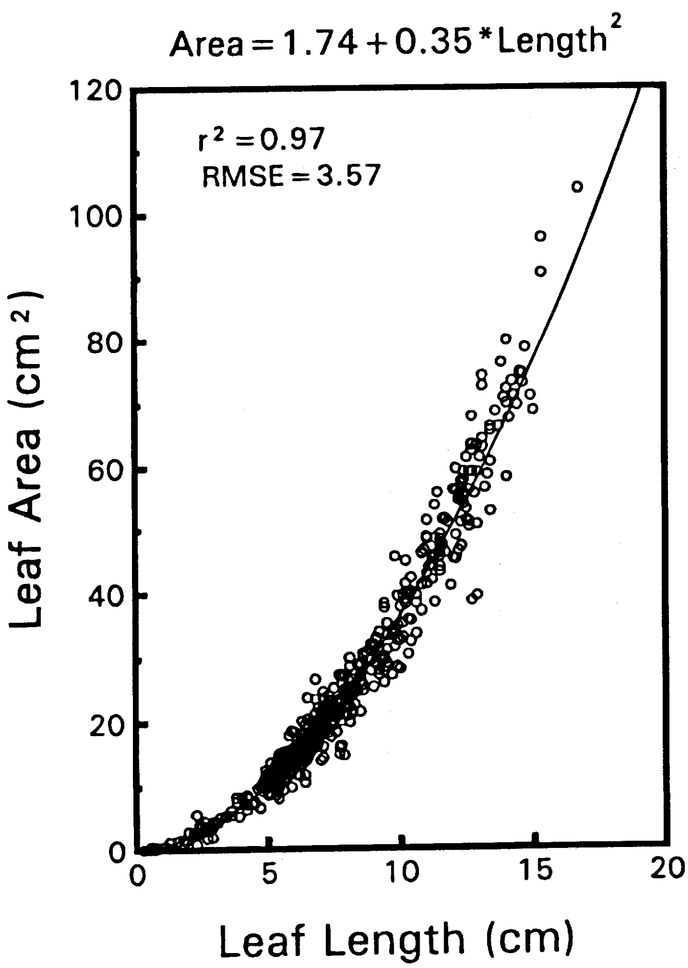

Fig. 3. Relationship between leaf area and length.

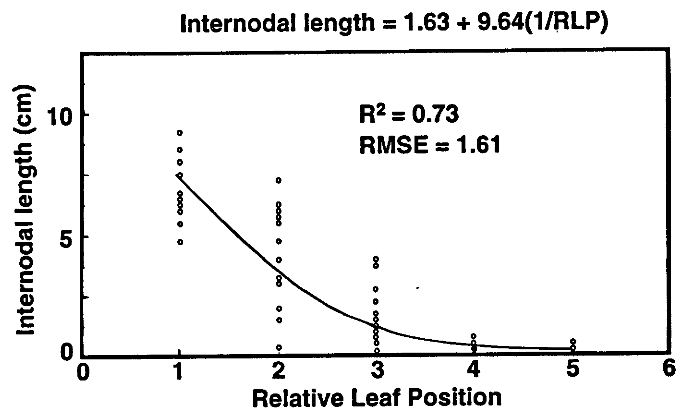

Fig. 4. Influence of relative leaf position on internodal length of flush 3.

Discussion

Apparently, leaf area for cherrybark oak was not affected by the development stage, position on the seedling, or the size of the leaves. Although the average leaf size for flush 3 was much greater than that for flush 1, the leaves from both flushes with similar length and width had similar leaf area. This fact seems to verify the assumption Persaud et al., (1993) indicated that leaves have invariant, genetically controlled shape and symmetry regardless of age and position.

For cherrybark oak seedlings, either length or width of the leaves can be used to predict leaf area, although length may provide a better estimate of the leaf area than width. Length was more closely related to leaf area and easier to measure than leaf width because of the shape of the cherrybark oak leaves.

The best estimate of leaf area was from the cross product of length and width. By combining the two independent variables, we increased the correlation between leaf area and leaf length x width. However, the improvement was slight compared to that between leaf area and length only. The improvement in r^2 was only 0.01. The difference between the two models is that one needs to measure both leaf length and width to gain that increase and therefore, it may not be worth the extra time required in actual studies.

Neither leaf fresh weight nor dry weight related to leaf area well. Leaf thickness may be a factor. However, measurement of leaf thickness has not be reported. Because collection of leaf weight requires destruction of the leaves, leaf weight probably should not be used to predict leaf area of cherrybark oak seedlings since destruction of the leaves may affect seedlings' physiological activities.

It should be pointed out that the models developed in our study were based on the limited number of seedlings in a greenhouse condition. Caution should be taken in applying these models to field studies since leaf shape and thickness may be affected by sun and shade leaves. Under greenhouse condition, however, the models can be tested with samples and used appropriately.

In conclusion, leaf length seems to be the most appropriate variable to predict leaf area. Leaf width can also be used. The use of the cross product of length and width can produce a slightly better result, but requires measurement of both leaf length and width.

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