

Leaf allometry of *Salix viminalis* during the first growing season

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Summary We established linear and exponential relationships between leaf area (A) and leaf length (L), leaf width (W), W^2 , L^2 and LW , in *Salix viminalis* L. Most relationships were significantly non-linear, but good fits were obtained with both linear and exponential models. The nonlinear relationship between A and LW differed for leaves from sylleptic and proleptic shoots. Leaves from sylleptic and proleptic shoots also differed in specific leaf area (area/weight). Leaf shape (width/length ratio and position of maximum leaf width) changed with leaf size and differed for leaves from sylleptic and proleptic shoots. Leaf area could be modeled adequately using implicit shape descriptions. A good fit was obtained when the basal and distal parts of the leaf were described as a parabola and an ellipse, respectively. The average area of single leaves and specific leaf area increased both along vertical profiles within shoots and during the growing period. Our results (1) indicate that nonlinear models should be used to estimate leaf area from linear leaf dimensions for plant species with leaves that vary in shape with leaf size, and (2) demonstrate the dependence of leaf characteristics on both sampling date during the growing season and spatial position in the canopy.

Keywords: allometric relations, isometric relations, leaf shape, leaf size, specific leaf area.

Introduction

In Swedish short-rotation forestry, fast-growing monocultures of basket willow (*Salix viminalis* L.) are grown at dense spacings on fertile soils to give a high production of biomass for use as fuel (Sirén 1983). The cultivation of basket willow as a commercial agricultural crop is expanding rapidly in Sweden, from 400 ha in 1988 to 9000 ha in 1993 (Johansson 1993). Although procedures for estimating stem biomass of *Salix* have been developed both for research and commercial purposes (Verwijst 1991, Telenius and Verwijst 1995), there have been few attempts to develop procedures for estimating leaf

area (Nilsson 1983) and little is known about single leaf allometry in willow.

Plant leaf area is directly related to light interception, transpiration and photosynthesis, and is considered to be the most important single determinant of plant productivity (Linder 1985). Reliable determinations of leaf area, however, are labor-intensive and costly, especially when time series are needed for fast-growing, high-yield plantations that change rapidly over time (Ceulemans et al. 1993). Indirect leaf area estimation methods based on light-interception measurements may be used at the stand level (Nobel et al. 1993), but these methods do not provide information about leaf area of individual trees, and they provide no information about the size and shape of single leaves.

Both leaf size and shape directly affect energy and mass exchange of the leaf because the thickness of the boundary layer limits the exchange of heat, water vapor and carbon dioxide (Parkhurst et al. 1968, Taylor and Gates 1970, Gates 1980). Therefore, measurements at the individual leaf level that define leaf shape and can be scaled up to obtain leaf area at the individual tree and stand levels (e.g., Ceulemans et al. 1993) would be useful in ecophysiological studies that span several scales.

The area of individual leaves can be estimated from non-destructive measurements of leaf width (W) and leaf length (L) (Wargo 1978, Ramkhalawan and Brathwaite 1990), and their ratios give some indication about the constancy of leaf shape with size. Humphries and French (1964) estimated leaf size by comparing leaves with simple geometric shapes, such as circles and ellipses.

We have studied leaf size, shape and specific leaf area (leaf area/leaf dry weight) of *Salix viminalis* L. The objectives of the study were to (1) establish relationships between linear leaf dimensions and individual leaf area; (2) determine the relationship between leaf shape and leaf size; and (3) assess the spatial variation of leaf characteristics within shoots and their dynamics throughout the first growing season.

Materials and methods

Site description

In spring 1994, 180 polyethylene barrels were put in the soil with their brims just above the soil surface, at a square spacing of 0.7×0.7 m, at the Ultuna field station, Uppsala ($59^{\circ}49'$ N, $14^{\circ}40'$ E, 5 m a.s.l.). Each barrel had a volume of about 220 dm^3 and contained a drainage tube at the bottom, connected with a pipe emerging at the soil surface, where surplus water could be extracted with a pump. The barrels were filled with washed quartz sand. In June 1994, one cutting of *Salix viminalis* (clone 78183, cf. Ager et al. 1986) was planted in each barrel. The barrels were covered with a lid that contained a hole through which the shoots grew. A drip irrigation system was used to add water and nutrients to each barrel. Nutrients and water were added daily in proportion to nutrient uptake, calculated on the basis of relative growth rates (Ingestad 1987).

From each cutting, one to five buds grew into proleptic shoots reaching a maximum length of about 1.5 m at the end of the season. Each shoot consisted of a stem with leaves and axillary buds. Some of these axillary buds developed into sylleptic shoots later in the season. Sylleptis implies that current-year axillary buds develop into shoots during continuous extension growth of the mother shoot (Kozłowski 1964). The leaves of *Salix viminalis* are ovate to lanceolate in shape and have entire margins.

Sampling and analyses

Four times during the growing season (July 26, August 15, September 5 and 26), seven plants with a minimum distance of 2 m from each other, were selected and one shoot was randomly chosen from each plant. From each of the chosen shoots, seven fully expanded leaves were sampled along a vertical profile from the base to the top at equal relative distances. On the last two sampling occasions, three leaves on each of seven sylleptic shoots were also collected. Leaf area was measured with a surface area meter (LI-3100; Li-Cor Inc., Lincoln, NE). Length (L), width (W) and the distance from the leaf base to the mid-vein point at the position of maximum leaf width (P) were recorded for each leaf. All leaves were oven-dried at 70°C for two days and then weighed.

The lengths of the leaf halves (divided at the position of maximum width) P for the basal part and $L - P$ for the distal part were used together with W to calculate leaf area as an ellipse (obtuse point) or a parabola (acuminate point) or a combination of these shapes.

Least squares regression was used in both linear and nonlinear models. The nonlinear regression procedure used an iterative least-squares method (Hartley 1961). The initial starting values for all parameters were set to 0.1 and the tolerance criterion for convergence was 10^{-5} units of magnitude of each parameter. In the regression statistics used, a correction for sample size and number of predictors was applied. The results of the regressions were assessed on the basis of comparisons of r^2 , uncorrected for sample size and number of predictors used in the model, and of the standard error of the mean (SEM).

Results

The area of single leaves (A) could be predicted with a linear model on the basis of leaf length (L) or leaf width (W) alone, with r^2 values of 0.97 and 0.94, respectively (Table 1). Still higher coefficients of determination were obtained by taking the squares of the linear dimensions or using the product of L and W (LW) as the predictor variable. When a nonlinear model was fitted, the estimated exponent (b) was significantly different from 1 in all but one of the cases, indicating that the relationships were intrinsically nonlinear. The extent of the decrease in standard error, resulting from using a nonlinear model instead of a linear model, was related to the deviation of parameter b from 1. When A was regressed on the product of L and W , the linear model gave a slight underestimation of the area of small leaves, and an overestimation of the larger leaves (Figure 1A). Thus, although a linear regression of A on L had a high coefficient of determination, considerable systematic errors resulted from a linear fit compared with a nonlinear fit (Figure 1B).

In both the linear and nonlinear models, differences between leaves from sylleptic shoots (S) and leaves from proleptic shoots (P) were taken into account by including a nominal variable for leaf type (Table 2). Comparisons of the parameter estimates and standard errors of the parameters of the linear and nonlinear models showed that the relationship between A and LW was nonlinear and that leaf type had a significant effect (b_1 differed significantly from zero) in the nonlinear model. This difference was not detected by the linear model.

Nonlinearity of the relationship between A and LW implies that leaf shape is changing with changing linear leaf dimensions. This was confirmed by inspection of the ratio of W to L (W/L), and the position of maximum leaf width (Figures 2A and 2B). Although these measures showed considerable scatter, regression analysis indicated significant differences between S -leaves and P -leaves. For both leaf types, leaves became relatively narrower with increasing length. The S -leaves were broader than the P -leaves, and the W/L ratio decreased faster with increasing leaf length for S -leaves than for P -leaves (Table 3). Furthermore, the position of maximum leaf width, which divides the leaf into basal and distal parts, changed with increasing leaf length. Maximum leaf width was almost in the middle of the shortest leaves, whereas longer leaves had a distal part that was four times as long as the apical

Table 1. The r^2 -values and standard error of the mean (SEM) for linear ($A = aD$) and nonlinear ($A = aD^b$) regressions of leaf area (A) on leaf dimensions (D). The dimensions are leaf length (L), leaf width (W) or a combination of the two.

Dimension	Linear model		Non-linear model		
	r^2	SEM	r^2	SEM	95% CI for b
L (cm)	0.972	1.62	0.992	0.84	1.55–1.65
W (cm)	0.941	2.36	0.982	1.30	1.96–2.15
L^2 (cm ²)	0.985	1.17	0.992	0.84	0.77–0.83
W^2 (cm ²)	0.982	1.30	0.982	1.30	0.98–1.08
LW (cm ²)	0.998	0.44	0.998	0.40	0.94–0.96

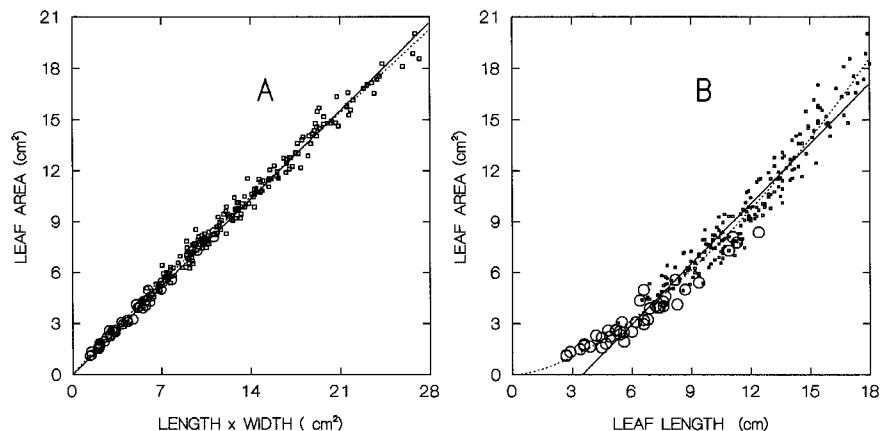


Figure 1. Relationships between leaf area and the product of leaf length and width (A) and between leaf area and leaf length (B), for leaves on sylleptic shoots (circles) and proleptic shoots (squares) based on a linear (solid line) and a non-linear (dotted line) fit. Deviations from a linear fit are small in the first case and larger in the second, but significant in both cases (see Table 1).

Table 2. Regressions of leaf area (A , cm^2) on the product of leaf length and width (LW , cm^2) and including a nominal variable (cat) for leaf type ($\text{cat} = 1$ for leaves on proleptic shoots, $\text{cat} = 2$ for leaves on sylleptic shoots), using a non-linear and a linear ($c = 1$) model: $A = (b_0 + b_1 \text{cat})LW^c$.

Model	b_0 (SE)	b_1 (SE)	c (SE)	r^2	SEM
Linear	0.74 (0.014)	0.006 (0.013)	1 (-)	0.998	0.44
Non-linear	0.906 (0.028)	-0.036 (0.014)	0.944 (0.007)	0.9984	0.39

part. The S-leaves had a relatively larger apical portion than the P-leaves, but the apical portion of S-leaves diminished faster with increasing leaf length than that of P-leaves (Table 3).

Differences in shape were successfully modeled by dividing each leaf into basal and distal parts and comparing each part based on simple geometric shapes such as ellipses and parabolas. In this way, we were able to estimate leaf area on the basis of leaf linear dimensions while taking account of the inherent changes in leaf shape with size. We tried six possible combinations of ellipses and parabolas, and found that a good fit was

obtained when the basal part of the leaf was described as a parabola and the distal part was described as an ellipse (Figure 3). This approach resulted in an overestimation of leaf area of $0.4 \pm 0.4\%$ (mean \pm standard error) for all sampled leaves.

We postulated that differences in leaf area of individual leaves along a vertical profile within shoots are related to differences in leaf size over time, because leaves that develop at the beginning of the season emerge on small shoots close to the ground, whereas later leaves develop higher up. This relation is illustrated by the response surface of leaf area to sampling occasion and relative height of the leaf on the shoot (Figure 4A). The smallest leaves on proleptic shoots were found close to the ground at the beginning of the season, whereas the largest leaves were found on distal shoot parts late in the season.

To estimate leaf area from leaf weight, we plotted specific leaf area (SLA, defined as the ratio between leaf area and leaf weight, M) against leaf area. The plot showed a large scatter, and there was a clear distinction in the bivariate means between S- and P-leaves (Figure 5). Although a linear model of A on M gave a good fit, the relationship proved to be nonlinear and differed between S- and P-leaves (Table 4). Much of the variation in SLA of P-leaves was explained by height (H) (Figure 6), which constituted a significant term in the non-linear regression model (Table 4). The change in SLA with

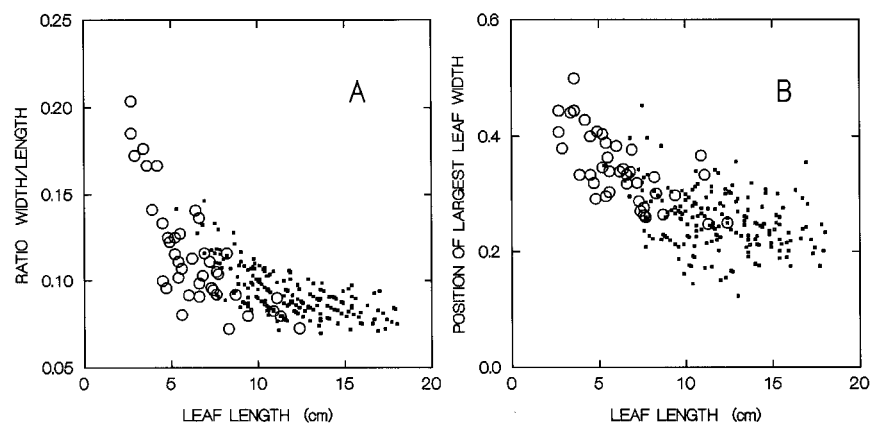


Figure 2. Relationships between leaf length and leaf width/length ratio (A) and between leaf length and the relative position of the widest part of the lamina along the midvein (B). These relationships describe changes in leaf shape with size, and show differences between leaves of sylleptic shoots (circles) and proleptic shoots (squares).

Table 3. Regressions of leaf width/length ratio and of maximum width position on leaf length for leaves on proleptic shoots (P) and sylleptic shoots (S), using the model: $Y = A + bL$, where Y is either the ratio or the maximum width position and L is leaf length.

Y	Type	a (SE)	b (SE)	r^2	SEM
Ratio	P	0.135 (0.003)	-0.004 (0.0003)	0.486	0.048
Ratio	S	0.184 (0.009)	-0.011 (0.001)	0.592	0.045
Position	P	0.329 (0.014)	-0.006 (0.001)	0.119	0.048
Position	S	0.450 (0.020)	-0.017 (0.003)	0.444	0.045

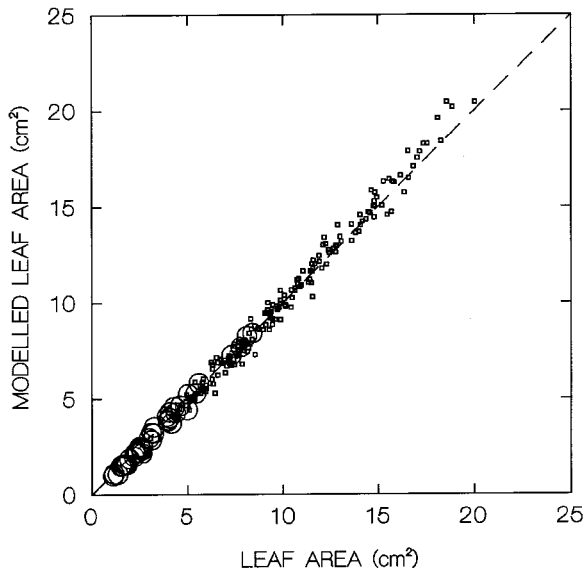


Figure 3. Relationship between measured and estimated leaf area when leaf shape was modeled implicitly with leaf size (the basal part of the leaf is described as a parabola and the distal part as an ellipse) for leaves on sylleptic shoots (circles) and proleptic shoots (squares).

height and time (Figure 4B) was similar to the change in A with height and time.

Discussion

We focused on leaf shape because changes in leaf shape with leaf size illustrate why nonlinear models may perform better than linear models for the estimation of leaf area from leaf linear dimensions.

When leaf area is estimated from a one-dimensional leaf characteristic such as length or width, the application of a linear model assumes that the two-dimensional area changes in direct proportion to the one predictor variable used, i.e., that there is an inherent isometric relation. It can be seen that this assumption holds true if other linear leaf dimensions are kept constant. This may be the case in many graminoids where leaf elongation can occur without changing leaf width; however, in many other plants, a change in leaf length accompanies a change in leaf width. If changes in one linear leaf dimension are proportional to changes in the other linear leaf dimension, and leaf shape is not otherwise changing, a linear model that uses the squared values of one of the dimensions will give a perfect fit. In most cases, however, relationships between leaf area and linear leaf dimensions and also the interrelations between the various linear leaf dimensions are not constant (e.g., Figure 2A). Therefore, if an inherent allometric relation is fitted with a linear model, systematic errors will occur, even if a high coefficient of determination is obtained. In our study, this was exemplified by the difference between a linear and a nonlinear model used to estimate leaf area from length and width and from length alone (Figure 1). Use of a single linear leaf dimension can be justified in cases where the number of measurements has to be restricted. When representative samples can be taken and the aim is to assess average values at the whole-plant level, linear models can be applied, but they may seriously limit interpretation of the data in terms of leaf size and shape variations.

The constant a in the linear model $A = aLW$ is often used to compare leaves of different clones, species or treatments (Kemp 1960, Ceulemans et al. 1993) and can be compared to the values of fixed geometrical shapes; e.g., a parabola and an

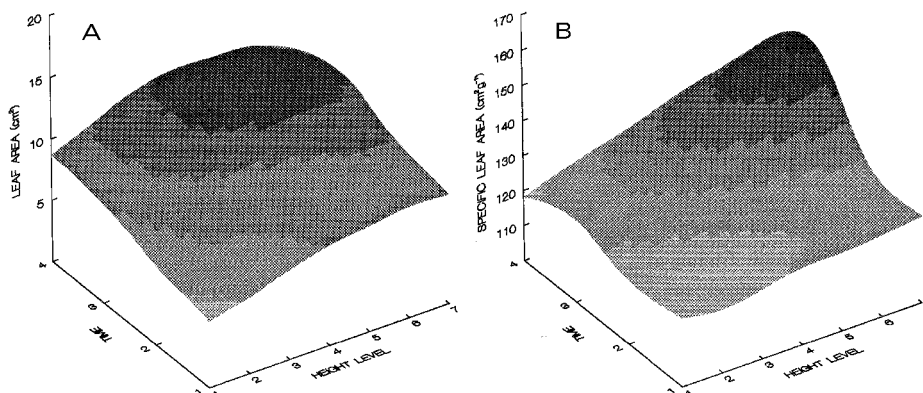


Figure 4. Changes in the response surface of leaf area (A) and of specific leaf area (B) with sampling time (July = 1 to late September = 4) during the season and with relative height on the shoot (shoot base = 1 and shoot tip = 7).

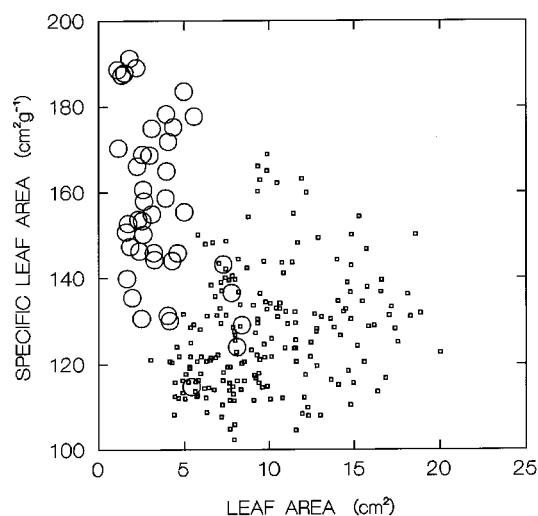


Figure 5. Relationship between specific leaf area and leaf area of leaves on sylleptic shoots (circles) and proleptic shoots (squares).

ellipse have values of 0.667 and 0.785, respectively. By dividing each leaf into basal and distal parts on the basis of the position of maximum leaf width and modeling leaf area as a combination of parabolic and elliptic parts, single-leaf values in the range between 0.667 and 0.785 are theoretically possible and may change systematically with leaf size. For leaves with a value beyond the range of 0.667–0.785, the procedure can be extended to cover the range from 0 to 1 by modeling leaf width as a function of leaf length, and integrating the resulting function to obtain the area. The disadvantage of an implicit shape description is the large number of variables that need to be determined for each leaf.

We found a nonlinear relationship between leaf area and leaf dry weight in *Salix viminalis*. Sharrett and Baker (1985) and Ma et al. (1992) also found that a nonlinear model best described the relationship between A and M for alfalfa (*Medicago sativa* L.) and peanut (*Arachis hypogaea* L.), respectively.

The differences in leaf area and specific leaf area (SLA) along a vertical profile and over a growing season showed that it is not valid to extrapolate results in space and time from a

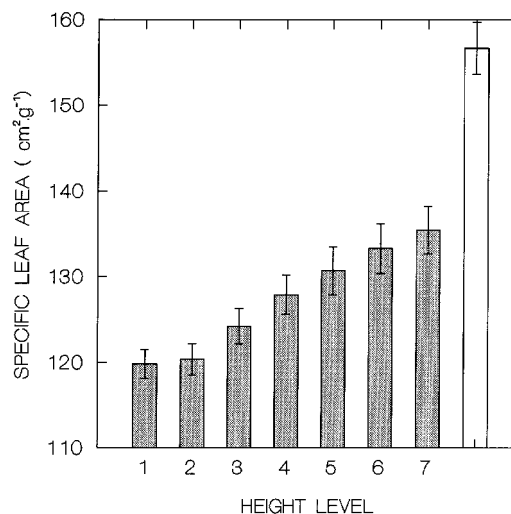


Figure 6. Relationship between specific area of leaves from proleptic shoots (shaded bars) and relative height of the leaf on the shoot. Leaves from sylleptic shoots (open bar) have a higher specific leaf area than leaves from proleptic shoots.

spatially and temporally restricted sampling. In fully developed stands, which may reach a maximum leaf area index of about 8 (Lindroth et al. 1994) and a height of about 7 m at harvest, strong vertical microclimatic gradients may lead to even greater differences in leaf characteristics than observed in this study. Reddy et al. (1989) concluded that 93% of the variation in SLA of cotton leaves (*Gossypium hirsutum* L.) could be accounted for by the light flux density within the cotton canopy. In their study, a high SLA was found at the bottom of the canopy and a low SLA was found at the top of the canopy. In our study, where the canopy was still open and provided little shade, the situation was reversed, and the variation in SLA was probably associated with a time-related factor such as growth stage or leaf maturity.

In willow, sylleptic shoots occur mainly during the first season (Brunkener 1988). The sylleptic shoots tend to wither in summer and eventually fall off. Development of sylleptic shoots during later years is rare. The finding that leaves from

Table 4. Regressions of leaf area (A , cm^2) on leaf dry weight (M , g) including a nominal variable (H) for relative height within the shoot ranging from 1 (shoot base) to 7 (shoot tip). P = leaves on proleptic shoots, S = leaves on sylleptic shoots, using the model: $A = (b_0 + b_1H)M^c$.

Model	Type	b_0 (SE)	b_1 (SE)	c (SE)	r^2	SEM
Linear	P + S	127.25 (0.845)		1 (-)	0.989	0.98
Non-linear	P + S	113.00 (4.90)		0.951 (0.018)	0.990	0.97
Non-linear	S	82.16 (7.70)		0.834 (0.027)	0.991	0.39
Non-linear	P	119.34 (6.09)		0.974 (0.021)	0.990	1.04
Non-linear	P	96.06 (4.49)	2.39 (0.34)	0.925 (0.017)	0.992	0.89

sytleptic and proleptic shoots differ with respect to size, shape and specific leaf area suggests that the functional aspects of these leaf types may also differ.

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