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ESTIMATION OF LEAF AREA OF SOYBEANS GROWN UNDER ELEVATED CARBON DIOXIDE LEVELS

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ABSTRACT

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Leaf area (LA) data are required for describing numerous canopy processes. However, determining LA for a crop is both time consuming and labor intensive, requiring a substantial investment of resources. The objectives of this study were (1) to develop statistical models for estimating LA of field-grown soybean (*Glycine max*) plants grown in open-top field chambers from measurements of destructive (leaf and top dry weight) and non-destructive (leaf number, plant height, and branch length) variables, (2) to examine the effect of CO_2 concentration on these statistical relationships, and (3) to test the applicability of such models to independent data collected under different experimental conditions. Predictive models of LA based on either branch length ($LA = 147.6 \cdot BRL^{0.635}$, CV = 11%) or top dry weight ($LA = 328.8 \cdot TDW^{0.731}$, CV = 12%) were found to have the lowest coefficient of variation about the regression line, to be unaffected by increasing CO_2, and to be reasonable predictors of LA under different growth conditions. Both leaf area per leaf and specific leaf area ratios changed with increasing CO_2 and growth conditions. Plant height was a poor predictor of LA.

INTRODUCTION

Leaf area (LA) data are required in mathematical models of numerous canopy processes, e.g., light interception (Burstall and Harris, 1983), photosynthesis (Heilman et al., 1977), transpiration (Enoch and Hurd, 1979), and growth rate (Warren-Wilson, 1981). Unfortunately, determining LA for a crop is both time consuming and labor intensive, requiring a substantial investment of resources. This problem is especially pertinent in our large

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scale field studies of soybean response to elevated atmospheric carbon dioxide (CO_2) (Rogers and Bingham, 1982) because of severe space limitations and the expense of operating open top fumigation chambers used for maintaining the various CO_2 levels (Rogers et al., 1983).

An alternative to direct measurement of LA is to develop mathematical formulas to estimate LA as a function of easily measured leaf properties, e.g., leaf width, length, dry weight, and numbers. This has been successfully done for a variety of crops, e.g., tobacco (Chen and Huang, 1970), sugarcane (Shih and Gascho, 1980), cotton (Wendt, 1967), alfalfa (Robinson and Massengale, 1967), sorghum (Shih et al., 1981), soybean (Fehr et al., 1971; Sivakumar, 1978), and barley (Ramos et al., 1983). The use of such predictive equations can reduce the overall sampling effort necessary to estimate LA, can increase the frequency of estimates (particularly when nondestructive variables are used), and can potentially be used on an independent data set obtained in another season or under different environmental conditions.

The objectives of the present study were (1) to develop statistical models for estimating LA of soybean plants grown in open-top field chambers from measurements of destructive (leaf and top dry weight) and nondestructive (leaf number, plant height, and branch length) variables, (2) to examine the effect of CO_2 concentration on these statistical relationships, and (3) to test the general applicability of these predictive models for use with independent data collected under different experimental conditions.

METHODS AND MATERIALS

Complete descriptions of the methods used are given in Rogers and Bingham (1982). The study was conducted on an Appling-Cecil soil association (both are clayey, kaolinitic, thermic Typic Hapludults) near Raleigh, NC, during 1981 and 1982. Soybeans (*Glycine max* (L.) Merr. 'Bragg') were grown in open top exposure chambers (Davis and Rogers, 1980; Rogers et al., 1983). These transparent chambers were 2.4 m high by 3.0 m in diameter and provided continuous exposure to CO_2 atmospheres. In 1981, plants were grown in two replicate series of six CO_2 concentrations: 332 (ambient), 428, 534, 623, 772, and 910 μmol mol^{-1} CO_2. In 1982, five CO_2 treatments with two replications each were used: 349 (ambient), 421, 496, 645, and 946 μmol mol^{-1} CO_2. The difference in ambient values between the 2 years was due to sampling error. In 1981, plants were grown in 16.5-l pots containing a 1 : 1 : 2 mixture (by volume) of sand, Metro Mix 220 (W.R. Grace Co.), and sandy clay loam at a density of 6 plants m^{-2}. In 1982, plants were grown directly in field soil in 1-m rows at a stand density of 15 plants m^{-2}. Five plants per treatment were harvested at days 14 (seedling), 49 (anthesis), and 84 (early pod-fill) from planting (10 July 81) for the potted plant experiment and at days 14 (seedling), 29 (mid-vegetative), 76 (early pod-fill), and 87 (mid pod-fill) from planting

(29 June 1982) for the field study. In both years buffer zones were maintained to avoid alteration of intraspecific competition during sequential harvests. After measuring height and branch length, plants were divided into leaves and stems. LA was measured with a leaf area meter (LiCor Model 3100) and separated into main stem leaf area (MLA) and secondary-stem leaf area (STLA). Dry weights were obtained after oven-drying at 55 ± 5°C for 72 h. The 1981 potted plant data were used to develop the LA models described below and the 1982 field data served as independent validation data for testing the generality of the models for use under different environmental regimes.

Linear and nonlinear regression analyses were used to examine the relationship of plant LA (MLA + STLA) to the independent variables NL (total number of leaves), HGT (plant height), BRL (sum of total branch lengths), LDW (leaf dry weight) and TDW (top dry weight, i.e., leaf + stem). Numerous models were explored for each relationship and, initially, fitted separately to data from each CO_2 treatment. Analysis of covariance was used to test for homogeneity of intercepts and slopes (Zar, 1974). Where appropriate, the resulting parameter estimates were examined for trends with increasing CO_2 and described statistically. Criteria suggested by Draper and Smith (1966) were used in assessing model goodness-of-fit: (1) the coefficient of determination (R^2), (2) the coefficient of variation (CV), i.e., the standard error of the regression expressed as a percent of the mean LA response, and (3) residual plots to examine constancy of model variance. The simplest model was favored in all cases. All tests of statistical significance were conducted at $P < 0.05$.

RESULTS

1981 pot data

Plant LA was proportional to the total number of leaves (NL) at each CO_2 treatment and sampling date in the pot experiment. The results of fitting the model LA = $a + b \cdot$NL to the separate CO_2 treatment data are given in Table 1. The intercepts were found to be nonsignificant and tests for homogeneity of slopes indicated a statistically significant effect of CO_2. Hence, these data were pooled and fitted with the slope set to vary linearly with CO_2 concentration as LA = $(a + b \cdot CO_2)$NL. These results are given in Table 1 and Fig. 1a. The leaf area per leaf ratio (LA/NL), increased from 87.9 cm^2 $leaf^{-1}$ at 332 μmol CO_2 mol^{-1} to 94.8 cm^2 $leaf^{-1}$ at 910 μmol mol^{-1} (Fig. 2a), an increase of 1.2 cm^2 $leaf^{-1}$ for each 100 μmol mol^{-1} increase. These data were somewhat variable as evidenced by the CV of 23%.

The relationship of LA to total branch length (BRL) and plant height (HGT) were both described with the power function LA = $a \cdot X^b$, where X = BRL or HGT. For BRL, the shape of the curve was concave down (i.e., $b < 1$) whereas for HGT the curve was concave up (i.e., $b > 1$). The

TABLE 1

Summary of parameter estimates for each regression model relating LA to NL, BRL, HGT, LDW and TDW. Based on 1981 potted plant experiment

Variable	Model	CO_2 level (μmol mol^{-1})	Parameter estimates		df	R^2	CV (%)
			a	b			
Number of leaves	LA = $(a+b\cdot CO_2)$NL	Pooled	83.9	0.012	180	0.97	23.2
Stem branch length (cm)	LA = $a\cdot$BRL^b	332	95.2	0.712	15	0.98	5.3
		428	113.2	0.686	15	0.98	6.8
		534	145.5	0.635	15	0.98	10.5
		623	120.4	0.670	15	0.97	8.4
		772	165.7	0.614	15	0.98	14.6
		910	130.9	0.652	15	0.98	11.7
		Pooled	147.6	0.635	90	0.97	10.9
Height (cm)	LA = $a\cdot$HGT^b	332	0.41	2.25	30	0.96	23.6
		428	0.23	2.37	30	0.94	30.1
		534	0.43	2.21	30	0.98	21.7
		623	0.10	2.54	30	0.98	20.3
		772	0.67	2.10	30	0.97	23.9
		910	0.27	2.32	30	0.98	20.3
		Pooled	0.45	2.20	180	0.97	23.9
Leaf dry weight (g)	LA = $(a+b\cdot CO_2)$LDW	Pooled	280.38	−0.087	180	0.98	16.9
Top dry weight (g)	LA = $a\cdot$TDW^b	332	324.4	0.702	30	0.94	18.4
		428	181.8	0.843	30	0.98	10.5
		534	284.5	0.728	30	0.96	19.7
		623	198.1	0.786	30	0.99	10.6
		772	190.9	0.808	30	0.99	11.9
		910	197.9	0.803	30	0.98	11.0
		Pooled	328.8	0.731	180	0.98	11.9

Degrees of freedom given are uncorrected total. BRL measurements were not taken on harvest day 14 (and five data points were missing) resulting in df = 15 per treatment.

results are summarized in Table 1. Tests for homogeneity of slopes were based on the log-linearized form of these models, i.e., log LA = log a + $b\cdot$log X, and showed no CO_2 effect on parameter estimates for either BRL or HGT. Thus, estimates for parameters a and b using the pooled data were obtained for each model (Table 1) and predictions of LA (by CO_2 treatment) based upon the BRL model (CV = 10.9%) and HGT model (CV =

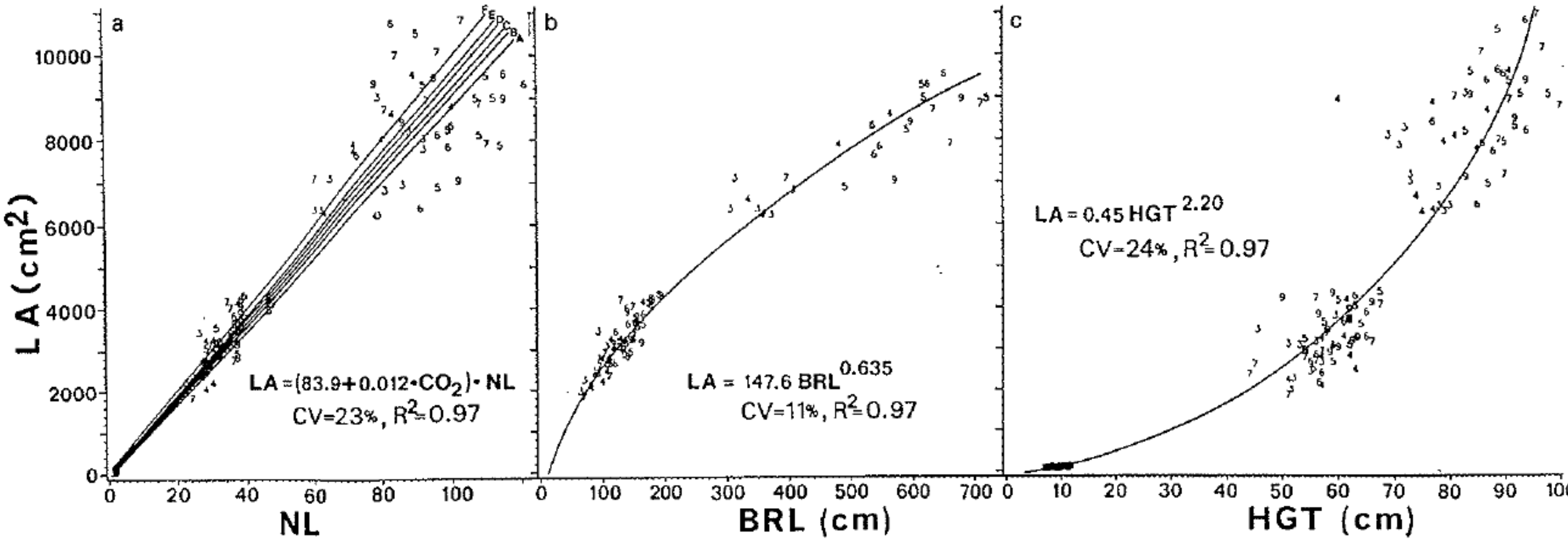

Fig. 1. Leaf area (LA) plotted as function of (a) total number of leaves (NL), (b) total branch lengths (BRL), and (c) plant height (HGT). Observed values of LA by CO_2 treatment are shown along with predicted lines. In (a), five lines are possible depending upon the CO_2 concentration (*A*=332, *B*=428, *C*=534, *D*=623, *E*=772, and *F*=910 μmol mol^{-1}). Numbers represent observed values from each of the CO_2 levels (*3*=332, *4*=428, *5*=534, *6*=623, *7*=772, and *9*=910 μmol mol^{-1}). Some observations are hidden. Clustering of data is due to the timing of sequential harvests.

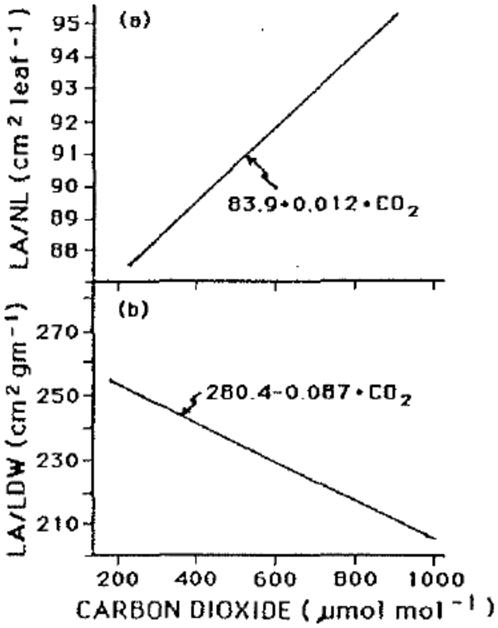

Fig. 2. Changes in (a) the leaf area per leaf ratio (LA/NL) (these are the slopes from Fig. 1a) and (b) the leaf area per unit dry weight ratio (LA/LDW) (these are the slopes from Fig. 3a) with increasing CO_2 concentration.

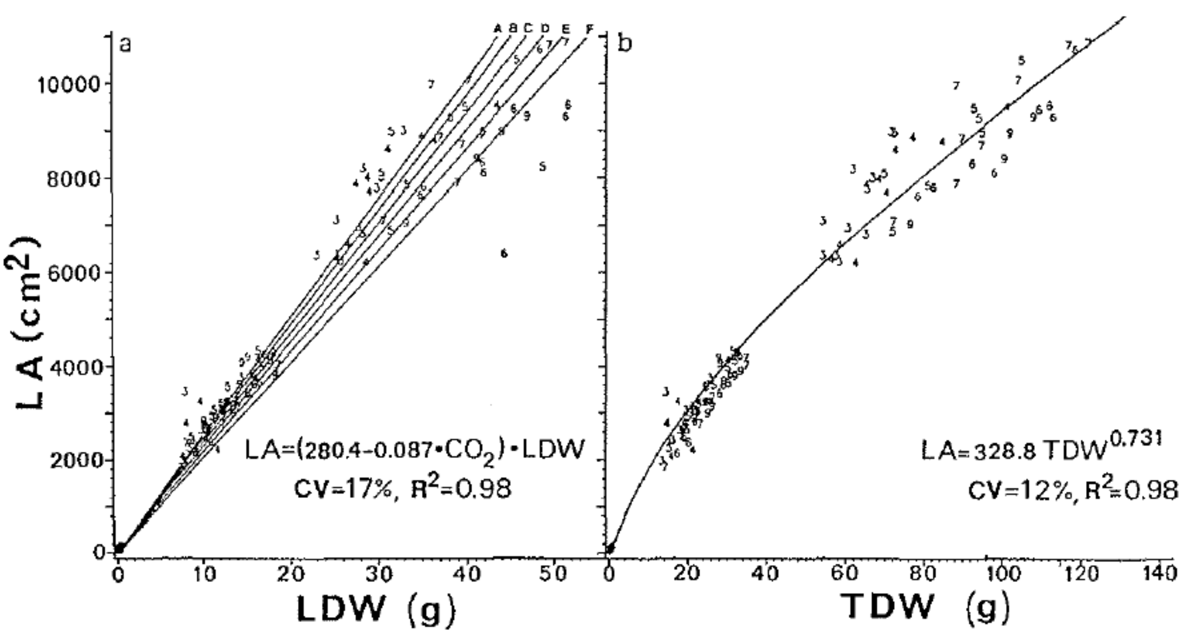

Fig. 3. Leaf area (LA) plotted as a function of (a) leaf dry weight (LDW) and (b) top dry weight (TDW). See Fig. 1 for explanation of symbols and lines.

23.9%) are shown in Figs. 1b and 1c, respectively. As with NL, the HGT model had a high variability about the regression line.

The specific leaf area, i.e., leaf area per unit leaf dry weight (LA/LDW), was examined by fitting the model LA = $a + b \cdot$LDW to each CO_2 treatment data (Table 1). While the intercepts were not significantly different from zero, the slopes were found to be statistically different, suggesting that specific leaf area declined with CO_2 and the model LA = $(a + b \cdot CO_2)$-LDW was fitted to the pooled data. Parameter estimates are given in Table 1 and the model is shown graphically in Fig. 3a. The value of −0.087 for parameter b in this model indicates a CO_2 effect on the prediction of LA from LDW measurements, i.e., an 8.7 cm^2 g^{-1} leaf weight decline per 100 μmol mol^{-1} increase in CO_2 (Fig. 2b). The ratio of total leaf area to top plant dry weight (LA/TDW) was examined in a similar manner to LDW. However, due to a strong nonlinearity in this relationship, the power model LA = $a \cdot TDW^b$ was the simplest equation found to provide an accurate description of these data. The parameter estimates for each CO_2 treatment are given in Table 1. No statistical differences in parameters were evident across the CO_2 levels and a pooled model was fitted (Table 1 and Fig. 3b).

1982 field data

The validation study focused on the extent to which the LA models developed from the 1981 plotted plant study were adequate for describing LA of 1982 field crops grown under different environmental regimes and the appropriateness of the functional form of the LA models (e.g. linear vs. nonlinear, etc.). With regard to the latter, we hypothesized that due to differences between the range of the variables collected between the 2 years (see Table 2), the functional form, in some cases, could have been appropriate but require a re-estimation of the parameters.

TABLE 2

Mean and range of variables used in 1981 and 1982 studies

Plant variable	Units	1981 Data		1982 Data	
		Range[a]	Mean	Range[b]	Mean
LA	cm^2	49—13023	4016	36—9880	2732
NL	—	1—154	43	2—63	18
HGT	cm	7—105	50	6—187	63
BRL	cm	64—897	268	0—847	189
LDW	g	0.1—69	17	0.1—36	8
TDW	g	0.2—162	37	0.2—111	26

[a] Data from day 14 to 84 after planting.
[b] Data from day 14 to 87 after planting.

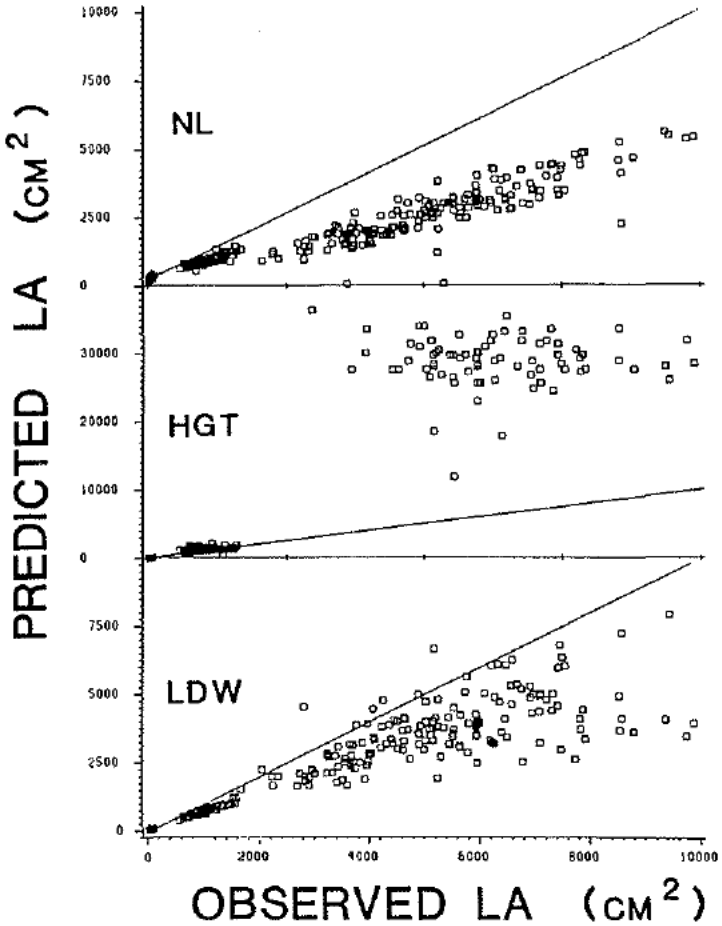

Fig. 4. Comparisons of predicted leaf area (LA) (models and parameter estimates developed from 1981 potted plant study) with observed LA (from the 1982 field study) from the models based on the independent variables: number of leaves (NL), plant height (HGT), and leaf dry weight (LDW). The solid line is the 45° line where observed and predicted values of LA are equal.

Results for the NL, HGT, and LDW models are illustrated in Fig. 4, which shows the observed (1982 field data) vs. predicted (models based on 1981 data) values of LA. In the case of NL, plant LA remained proportional to NL but the constant of proportionality increased substantially from the 1981 data; hence, the predictions fall below the 45° line in Fig. 4. Refitting the model to the 1982 data gave the following: LA = (154.73 + 0.017· CO_2)NL (R^2 = 0.97, CV = 23.6%). Although the overall leaf area ratio (LA/NL) increased in 1982, the effect of CO_2 on this ratio was found to be statistically unaffected (0.017 (cm^2 mol^{-1}) μmol^{-1} CO_2 compared to 0.012 (cm^2 mol^{-1}) μmol^{-1} for the 1981 data). The CV of 23% was the same as the 1981 model.

The LA model based on plant height (HGT) was satisfactory for the early field harvests (days 14 and 29) but wholly inadequate for the later dates (Fig. 4). In fact, the high variability in these 1982 field data for HGT did not warrant further model description. Predicting LA from LDW was also not very successful (see Fig. 4). An increase in the specific leaf area in the field grown crop is evident in Fig. 4 (i.e., the predicted values fall below the 45° line). Refitting the model to the 1982 data resulted in the following: LA = $(353.98 - 0.054 \cdot CO_2) \cdot$ LDW (R^2 = 0.93, CV = 34.6%). The specific leaf area declined with increasing CO_2, a result similar to that found in the 1981 study, but at a statistically significant lower rate (a 5.4 cm^2 g^{-1} leaf weight per 100 μmol mol^{-1} decrease in CO_2 compared to 8.7 in the 1981 study). These results must be viewed cautiously, however, because of the high variation in these data as reflected by the large CV for the model.

The results of using the BRL and TDW models to predict field LA are illustrated in Fig. 5. Both models provided good predictions of LA (as evidenced by the scatter of points around the 45° line) across each of the CO_2 treatments. While the BRL model underestimated LA at the lower range of LA values (Fig. 5), the TDW model provided excellent predictions of LA at lower ranges. At the later stages of growth, it can be seen from

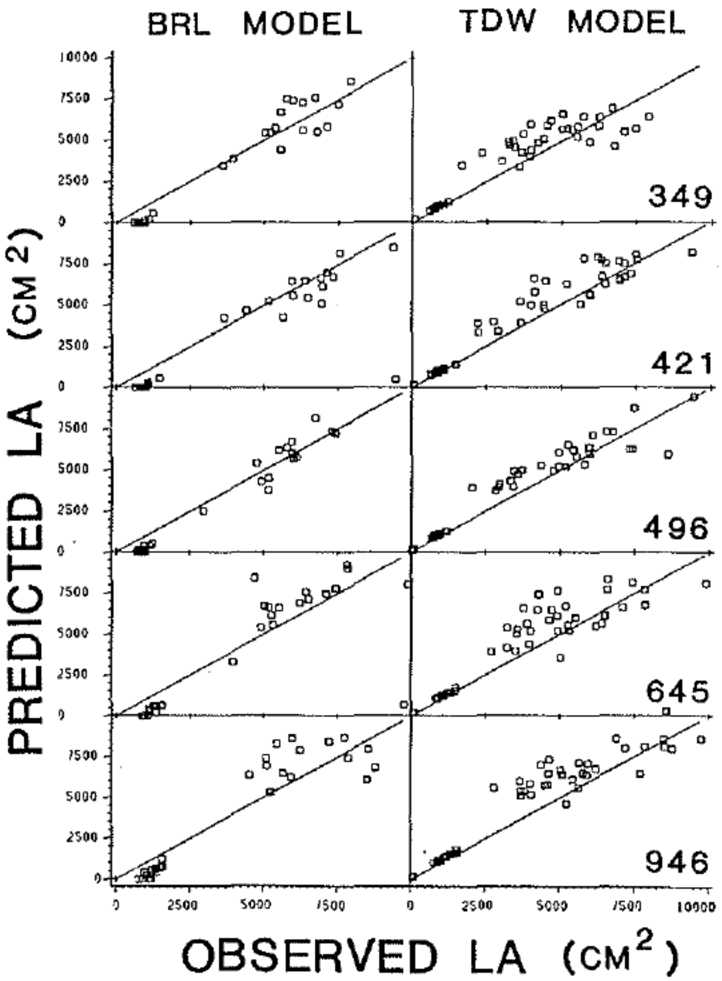

Fig. 5. Comparisons of predicted leaf area (LA) (models and parameter estimates developed from 1981 potted plant study) with observed LA (from the 1982 field study) from models based on the independent variable branch (BRL) and top dry weight (TDW). The solid line is the 45° line where observed and predicted values of LA are equal. Results from each of the five CO_2 treatments are shown.

Fig. 5 that there was little difference in the models. Hence, in cases where BRL is large (BRL > 60 cm), because of the ease of determination of BRL, it would be the preferred model. Clearly, where BRL is small, such as in young stands or for situations where branching is suppressed, BRL cannot be used to predict LA. A wide range of effective plant architectural densities occurred in this study due to the various CO_2 treatments and to the differences in planting densities in the pot (6 plants m^{-2}) and field (15 plants m^{-2}) experiments. The range of intraspecific competition represented illustrates the positive correlation between branch length and leaf area. The photomorphogenic effects that result in an increase in leaf area also increase branch length. Our observations of leaf/branch relationships at different planting densities invariably suggest that leaf area varies in a predictable manner with branch length.

DISCUSSION

The results of this study suggest that total leaf area (LA) of a field grown soybean plant grown under the set of conditions described here, can be best estimated from simple nondestructive measurements of total branch length (BRL) or from top dry weight (TDW). The relationships of LA to BRL and TDW, described by power functions, appear to hold reasonably well under different experimental conditions and appear to be little affected by increasing levels of CO_2. Conditions under which branch length would be surpressed are not addressed in this study. Leaf dry weight (LDW) and total number of leaves (NL) are also potentially useful for estimating plant LA, but models based on these variables tend to have higher variability and must be recalibrated for plants grown under different conditions. This is due to changes in the specific leaf area (LA/LDW) and leaf area per leaf (LA/NL) ratios. Ogbuehi and Brandle (1981) also reported differences in these ratios for soybean plants grown under varying environmental conditions. Plant height is not recommended as an estimator of LA because of the high degree of sampling error.

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