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Relationship between Net CO_2 Assimilation and Dry Weight Accumulation in Field-Grown Tobacco

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ABSTRACT

To assess the variability of net photosynthetic CO_2 exchange per unit leaf area and to construct budgets for stands of field-grown tobacco (*Nicotiana tabacum*, Connecticut Broadleaf), a number of short-time measurements were made on all available leaf positions on two varieties using a hand-held transparent chamber for conducting gas exchange measurements on leaves. Measurements of net CO_2 exchange were carried out on 18 separate days during a 35-day period, beginning 22 days after the seedlings were transplanted to the field. Gas exchange assays on leaves were conducted under ambient conditions of temperature and light intensity at all times of day. Solar radiation was monitored throughout the period, and losses of respiratory CO_2 from stems, roots, and leaves (in the dark) were estimated. A simple model was proposed to relate daily total CO_2 input to irradiance and total leaf area. The total leaf area was assumed to be a function of day number. Dark respiratory losses accounted for 41% to 47% of total CO_2 assimilation. Analysis of variance indicated that the two varieties were not significantly different in whole plant rate of CO_2 fixation per unit of leaf area. CO_2 input was closely associated with leaf area within each variety. Throughout the experiment, the difference between the two varieties in total leaf area per plant was the largest single factor in determining net CO_2 inputs. The cumulative dry weight increase for each variety was similar to the prediction of net dry matter input obtained by gas exchange measurements, thus confirming the close relationship between total plant net CO_2 assimilation and dry weight yield.

We have been involved in a continuing effort to modify genetically the biochemistry of C_3 plants to produce tobacco mutants with decreased photorespiration and greater than normal rates of net CO_2 assimilation per unit of leaf area (1, 2, 10, 16). To confirm that varieties with superior rates of net photosynthesis under controlled conditions of the laboratory or greenhouse perform similarly in the field, an accurate yet convenient method of measuring net CO_2 uptake under field conditions was sought. Most recently, we adapted the CO_2-depletion system described by Clegg *et al.* (5, 13) to tobacco leaves in the field. It is based on the short-time measurement of the decrease in CO_2 concentration in a portable, hand-held transparent chamber clamped over the leaf. This method permitted the convenient assay of large numbers of samples, and the results proved to be reliable and reproducible.

It became apparent from our experience and from that of others that a limited number of instantaneous measurements of rate of CO_2 fixation per unit of leaf area are frequently not highly correlated with yield (18). Nevertheless, integrated seasonal assays of net CO_2 exchange should be closely related to dry weight accumulation. A limited number of investigations have been described in which a carbon budget has been approached under field conditions. An estimate of the carbon assimilation derived from CO_2 and the accumulation of dry matter in barley was carried out by Biscoe *et al.* (3). They found good agreement during an 11-week period. High correlations in field experiments between integrated net CO_2 exchange and dry weight accumulation have been described for soybean (4), wheat (11), sorghum (9), and maize (15).

Ideally, one would like to compare otherwise isogenic lines of the same species that differ only in the rate of net CO_2 exchange per unit of leaf area, perhaps because photorespiration is regulated, to determine the relationship between net mg $CO_2/dm^2 \cdot h$ and total dry weight accumulation. Unfortunately, such plant material is not yet available. Instead, we compared the carbon budget of two varieties of Connecticut Broadleaf tobacco with similar yet nonidentical genetic backgrounds. One variety, possessing resistance to tobacco mosaic virus, yielded somewhat less than the parent variety (G. S. Taylor, personal communication). We wished to determine: (a) the extent of variability under field conditions in net CO_2 exchange per unit leaf area in order to find what differences in such rates could be detected; (b) what other variables such as leaf area and dark respiration most affect the accurate estimation of a carbon budget; and (c) whether one could determine from a carbon budget why one variety produced more dry weight than the other. This investigation has demonstrated the usefulness of the CO_2-depletion method of assaying net CO_2 exchange in field studies, and clearly established the close relationship between net CO_2 exchange and dry weight accumulation.

MATERIALS AND METHODS

Plant Material. *Nicotiana tabacum* (Connecticut Broadleaf) 73-6-1 (variety 1) and 73-12-1 (variety 2, homozygous mosaic resistance derived from *N. glutinosa*) were obtained from the Valley Laboratory, The Connecticut Agricultural Experiment Station, Windsor, CT. The lines were chosen because they had similar genetic backgrounds but differing yields during field trials (G. S. Taylor, personal communication). Seedlings were grown in a greenhouse for 1 month prior to transplantation to the field on May 28, 1981. The field plot was fertilized with 1500 kg 10-10-10 (N-P-K) per ha. About 200 plants of each line were planted alternately throughout the 0.2 ha plot at 1.8 m spacings. Rainfall provided adequate moisture during much of the season. However, a brief drought necessitated irrigation with 2.5 cm water on day 28.

To facilitate growth analysis and assessment of photosynthetic CO_2 input, axillary buds and flower buds were removed as they appeared on all test plants. Growth and photosynthesis measurements started 22 d after transplantation to the field on June 19, 1981 (day 1) and ended as senescence approached on July 23 (day 35).

Sampling. Five different test plants from each variety were selected daily for CO_2 fixation measurements according to a Latin

square procedure designed to sample all regions of the plot. Leaves were generally assayed for net CO_2 exchange over an 8 h period each day beginning at 0600, 0900, or 1200 (EDT). Sunrise was about 0500 and sunset about 2000 (EDT) during the 35 days. Leaves were assigned position numbers starting from the ground up. The method of selection of leaves for measurements of CO_2 fixation was designed to be representative with regard to replicate plants and leaf positions. One leaf per plant in turn was assayed for net CO_2 exchange (mg $CO_2/dm^2 \cdot h$) using the procedure described below. The lowest leaf was sampled first, then the next until 10 leaves on 10 different plants had been assayed. When the uppermost leaf on a plant was too small for measurement, the sequence of leaf selection was started over beginning at the lowest leaf. The second sequence of 10 assays was begun by skipping one leaf position to avoid assaying the same leaf on a given plant more than once. This procedure was performed six times (a total of 60 assays, 30 per variety) during each day so that varying numbers of replicate measurements were performed on all currently represented leaf positions in the stand but on different plants. Leaves were selected and assayed for CO_2 fixation without altering, insofar as possible, the leaf orientation, and irrespective of the physical condition of the leaf or its exposure to light. Measurements of growth and photosynthesis by the stands are expressed on a per plant basis.

Growth Analyses. Once each week during the course of the 35-d experiment, plants from each part of the plot were harvested and separated into leaves, stems, and roots. Leaf areas were measured with a LI-3000 Portable Area Meter (Li-Cor Instruments, Lincoln, NB). Plant material was dried at 80°C in a forced draft oven to obtain dry weight. Stem diameter values served as a nondestructive index of growth and leaf area (12), and were obtained by averaging two measurements made at 90° angles 2 to 4 cm above ground level with a vernier caliper. Stem diameter measurements were taken on plants used for growth analyses as well as on plants used in CO_2 exchange assays.

CO_2 Exchange Measurement. Details of the CO_2-depletion method and associated apparatus employed in this study were similar to those described by Clegg *et al.* (5, 13). The hand-held photosynthesis chamber consisted of two Plexiglas boxes (8.8 cm per side) open on one side and fastened together with a hinge with open sides facing each other much like open jaws so that a portion of a leaf would be enclosed in the chamber. The area of the enclosed portion was 0.731 dm^2. A closed-cell foam rubber gasket covered the edges of the jaws of the chamber and protected the leaf from injury when the chamber was closed. A locking bar was positioned to insure a tight seal at time zero. Normally, the top and bottom halves of the chamber were totally separated from each other by the tobacco leaf, hence photosynthesis by the upper and lower surfaces of the leaf was measured separately. Two small, dry cell battery-operated fans kept the air within the two halves of the chamber well mixed. Wind speed at 0.5 cm above the leaf surface was about 150 cm/s. During a typical assay of CO_2 uptake by a tobacco leaf, after closing the chamber, 5.0-ml samples of the enclosed gas were withdrawn with 10 cc plastic syringes (Becton, Dickinson) through a rubber septum at 2 and 22 s from the bottom half, and at 4 and 24 s from the top half. The 2 to 4 s delay resulted in greater reproducibility in the measurements probably due to complete mixing of air within the chamber. Syringes were stored with the needles inserted into rubber stoppers to retard leakage during the 10 to 60 min which elapsed before analysis of the CO_2 contents of the gas samples were conducted. Errors due to leakage by the syringes during this period were negligible.

The CO_2 analysis system consisted of a Beckman model 865 Infrared Analyzer operating in the absolute mode. Prepurified N_2 flowed at a velocity of 500 ml/min first through a soda lime filter to remove any traces of contaminating CO_2. The sample (5.0 ml) was then injected into the gas stream, passed through silica gel to remove H_2O, then through a MF-Millipore filter (type RA, 1.2 μm pore size) to remove particulate material, and finally to the sample cell of the IR analyzer. Prepurified N_2 continuously flowed at approximately 100 ml/min through silica gel and the reference cell. Components of the sample processing system were connected with 1.0 mm (i.d.) stainless steel tubing and Swagelok fittings. Sample peaks generated by the IR analyzer were integrated and printed out as $\mu l/l$ CO_2 by a Hewlett-Packard model 3390A Reporting Integrator. The system was calibrated with primary standard grade CO_2 in air (301 μl CO_2/l air) (Matheson Gas Products). The instrument response was linear *versus* CO_2 concentration in the range used. The rate of CO_2 exchange was calculated from the observed rate of change in CO_2 concentration based on a chamber volume of 0.681 liter using the ideal gas equation.

Measurements of Respiratory CO_2 Evolution. Details pertaining to CO_2 production due to respiration by stems, roots, and leaves (at night) are included in the legends to the appropriate figures and tables. Analyses of gas samples for CO_2 content in these experiments were performed using the apparatus described above.

Miscellaneous. Total solar radiation (cal/$cm^2 \cdot$min) was continuously monitored on a Recording Pyrheliometer (Belfort Instrument Co., Baltimore MD) located near the field plot. Regression analyses and analyses of variance were performed on a Wang 2200 computer.

RESULTS

Net CO_2 Exchange and the CO_2-Depletion Technique. Figure 1 shows the time course of CO_2 uptake by an attached, illuminated tobacco leaf under field conditions enclosed in the transparent chamber. A 20-s assay interval resulted in 15% to 20% depletion of the normal CO_2 concentration in the chamber during vigorous photosynthesis. Experiments in the laboratory with excised, greenhouse-grown tobacco leaves at high irradiance indicated that depletions of CO_2 to the extent normally encountered in these experiments might lead to an average underestimation of CO_2 input over the season by as much as 8%. Since the relative rate of change of photosynthetic rate with CO_2 concentration at atmospheric CO_2 levels is reduced at the lower irradiances encountered in the field by those leaves shaded by upper leaves (7), this error would be diminished somewhat. Overestimation of the mg $CO_2/dm^2 \cdot h$ measured on single leaves could occur due to decrease of the boundary layer diffusive resistance over the leaf surface by the air movement (about 150 cm/s) induced by the battery-powered fans in the chamber during assay. The significance of this latter error to the overall carbon economy cannot be assessed precisely but is certainly less than 10%. This overestimation would

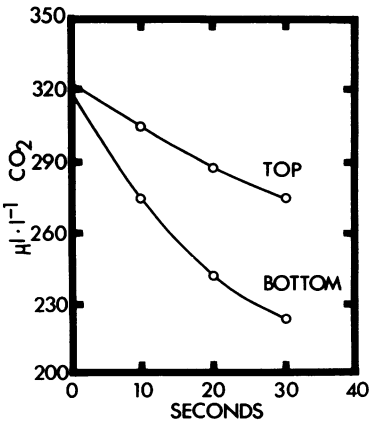

FIG. 1. Time course of CO_2 depletion using the technique described in "Materials and Methods" for estimation of the rate of photosynthesis by a leaf of field-grown tobacco. The rates of CO_2 uptake by the upper and lower surfaces of the leaf were 8.6 and 14.3 mg $CO_2/dm^2 \cdot h$, respectively.

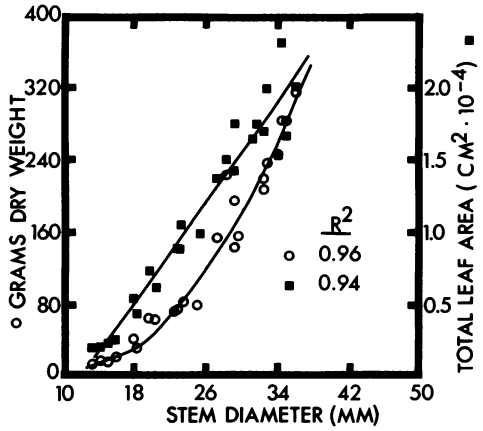

FIG. 2. Relationship between total dry weight (○), total leaf area (■), and stem diameter for field grown tobacco (variety 1). Values of r (R, correlation coefficient) were obtained from first- and second-order polynomial regression analyses of total leaf area and total dry weight *versus* stem diameter, respectively. Computed SE of the estimates were 19.9 g and 16.68 dm^2 for total dry weight and total leaf area, respectively. Similar regressions were employed in the analysis of growth and CO_2 exchange by variety 2 (not shown).

occur only under very still conditions (*i.e.* early morning hours) or with some lower leaves which are well sheltered by the rest of the canopy. The reported rates of CO_2 input have not been corrected for CO_2 depletion nor for changes in the boundary layer diffusive resistance. The short assay period employed during these measurements precluded any appreciable temperature rise inside the chamber. Analysis of CO_2 concentrations in gas samples with the IR system described gave quite reliable results. Replicate injections of standards agreed to within 1% to 2%.

A potentially important source of error was leakage of atmospheric CO_2 around the foam rubber gaskets into the photosynthesis chamber during the assays. In preliminary experiments, the closed chamber was flushed with N_2 and the rate of appearance of CO_2 from the atmosphere was measured. These experiments indicated that such leakage would lower the observed photosynthetic rate by only 1%. Hence, the reported values have not been corrected for chamber leakage.

Growth Analyses. Measurements of stem diameter at the base of the plants for several tobacco cultivars were highly correlated with the total leaf areas (12). We have confirmed that the stem diameter (measured always at the base of the plant) provides a convenient, nondestructive approximation of total leaf area, and have extended this correlation to total dry weight (Fig. 2). Similarly, a strong correlation was found between the dry weights of either leaves, stems, or roots *versus* stem diameter (data not shown). In addition, leaf area at any given leaf position of a plant was related to the stem diameter at the base of the plant by a second or third order polynomial function. Relative SE of the estimate for these latter regressions were lowest for the larger leaves found at positions 6 through 18 ($r > 0.90$). Considerable variation, which was unaccounted for by regression analyses, occurred in smaller leaves found at positions 3 through 5 and 19 and 20. These leaves, however, contributed little to the overall carbon economy of the plant.

Measurements of photosynthetic CO_2 exchange and growth spanned that part of the season during which 90% of the total dry matter was produced (Fig. 3). By day 35, all plants were showing signs of senescence. Although the two varieties differed by only 10% to 15% in total dry weight during the season, a two-way analysis of variance of the daily total dry weight measurements for the two varieties indicated that their respective mean total dry weights were significantly different ($F = 27.91$, $P < 0.001$; 1, 25, and 25 degrees of freedom for the varieties, days, and error, respectively).

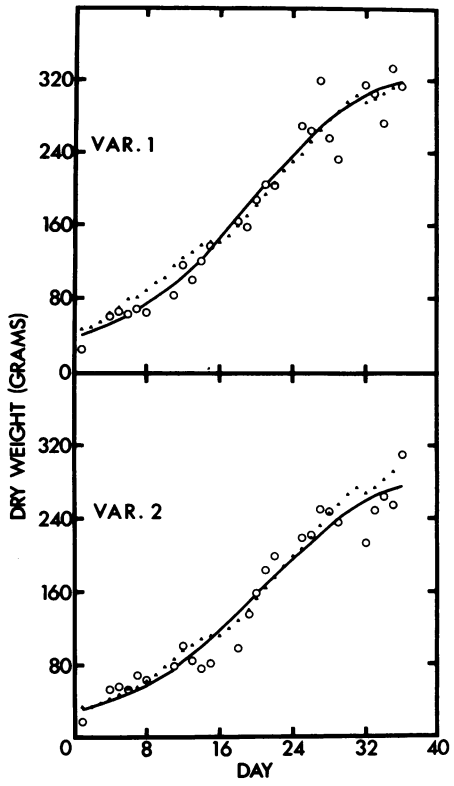

FIG. 3. Measured growth (dry weight) and predicted cumulative CO_2 assimilation by stands of field-grown tobacco (varieties 1 and 2) over a 35-d period. Measurements of stem diameter on five plants of each variety on each of the days shown were used to estimate the total dry weights of the respective plants (Fig. 2). Each of the points (○) shown is the arithmetic mean of the five estimates of dry weight. Growth measurements (day 1) commenced 22 d after transplantation of the seedlings to the field. Solid lines shown are third-order polynomial fits to the observed data obtained from regression analyses ($r^2 = 0.96$ and 0.94 for varieties 1 and 2, respectively). Total dry weight per plant for each variety of day 1 was estimated by assuming that growth during the first 12 d of the experiment followed an exponential function (relative growth rates of 0.086 and 0.087/d for lines 1 and 2, respectively). Cumulative CO_2 assimilation (Table IV) for each day (▲) was computed assuming a per plant dry weight of 40.3 and 32.1 g on day 1 for varieties 1 and 2, respectively. Total daily CO_2 input per plant was determined from *in situ* measurements of leaf photosynthesis. Respiratory CO_2 losses from roots, stem, and leaves (at night) were subtracted to yield net CO_2 incorporation. The carbon of the bulked plant dry matter was assumed to be at the oxidation level of carbohydrate ($[CH_2O]$), so the values of net CO_2 incorporation were multiplied by 0.68 for comparison with dry weight increases. See text for further details.

Table I shows that no significant difference was observed between the varieties regarding the seasonal mean ratio of total leaf area to total dry weight. Figure 4 shows that although the ratio of total leaf area to total dry weight varied over the course of the season, the varieties did not differ significantly in this respect. Slight, but statistically significant, differences were found between the two varieties with respect to the distribution of dry matter among the plant parts. These differences in distribution probably did not significantly affect the relative yields of the two varieties (see below).

Photosynthetic Carbon Input into Single Leaves. Photosynthesis by single leaves as measured with the CO_2-depletion technique and expressed as mg $CO_2/dm^2 \cdot h$ was quite variable. Much of this variation could be ascribed to environmental effects (especially

Table I. *Distribution of Dry Matter between Leaves and Stems in Two Varieties of Field-Grown Tobacco*

Once a week for 5 weeks, five plants (including roots) of each variety were harvested, dried, and weighed as described in "Materials and Methods." Three-way analyses of variance were performed on arrays of data constructed according to days, varieties, and replicates (degrees of freedom associated with varieties = 1, replicates = 4, days = 4, and error = 14, two missing observations).

	Total Leaf Area / Total Dry Wt	Total Leaf Dry Wt / Total Dry Wt	Stem Dry Wt / Total Dry Wt
	dm^2/g	g/g	
Variety 1	0.98	0.70	0.18
Variety 2	1.01	0.73	0.16
F	1.33	7.03[a]	6.10[a]

[a] Significant at P = 0.05 level.

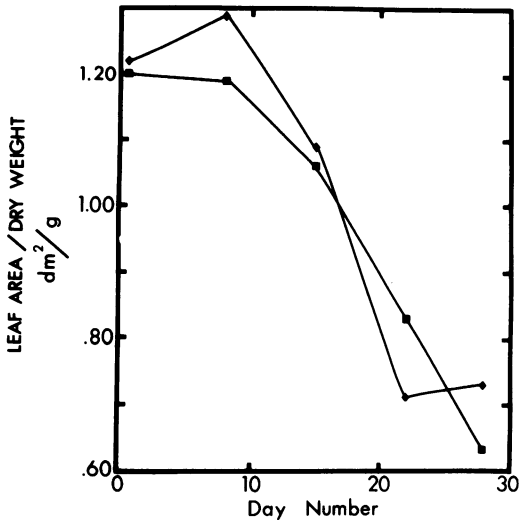

FIG. 4. Ratios of total leaf area to total dry weight *versus* day number for variety 1 (■) and variety 2 (◆). Each point is a mean of five determinations. Leaf area and dry weight determined as described in "Materials and Methods." Differences between the mean values shown for the two varieties are statistically significant (P = 0.05) only for day 22. Seasonal mean ratios of total leaf area to total dry weight are not significantly different (Table I). However, the average total leaf area per plant was greater in variety 1 than in variety 2 at all times during the experiment as predicted by the consistently greater total dry weight of variety 1 (Fig. 3). Greater leaf area is the major reason for greater CO_2 input by variety 1 during the 35-d experiment.

irradiance), plant age, and shading by other leaves in the canopy depending on leaf position and orientation (Table II). In our study, every effort was made to estimate the existing photosynthetic rate and to avoid biasing estimates of rates of whole plant CO_2 input with respect to any given leaf position. Likewise, any assay of CO_2 input by a single leaf was performed without alteration of leaf angle or exposure to sunlight within the canopy.

An estimate of total leaf CO_2 input per hour was made by multiplying the observed mg $CO_2/dm^2 \cdot h$ by the leaf area as estimated by the stem diameter at the base of the same plant (see above). The mean rate of CO_2 input into the stand (expressed on a per plant basis) at any given leaf position was, in turn, obtained by averaging from one to four estimates of total leaf photosynthetic rate taken on different leaves but at the same leaf position on five test plants during the sampling period (usually 8 h).

Analysis of CO_2 Input into the Leaf Canopy. The modified Latin square procedures employed in selecting both test plants and leaves for measurements of rates of CO_2 input were designed to sample the variability in rates of CO_2 input per leaf by all of the leaf positions currently represented in the stand. Summation of the average inputs in all leaf positions provided an estimate of the mean rate of CO_2 fixation by the stand expressed on a per plant basis. A distinct advantage of our experimental approach was that characteristics of leaf canopy photosynthesis could be examined without interference caused by the presence of stems or fruits which may occur with whole plant chambers used with tobacco or with other crop plants. Also, the daily relative contribution of CO_2 by groupings of related leaf positions to the total CO_2 fixation by the canopy could be assessed.

Table II. *Net Photosynthetic CO_2 Fixation Rates at Different Leaf Positions in a Stand of Field-Grown Tobacco on an Overcast and on a Sunny Day*

Measurements of photosynthesis on individual leaves of variety 2 were made using a CO_2-depletion method of 20 s duration (see "Materials and Methods"). The number of determinations at each leaf position are shown in parentheses. The values of photosynthesis shown were obtained on an overcast day (day 7, 239 cal/cm²·day) and on a sunny day (day 20, 643 cal/cm²·day) and illustrate the strong effect of light intensity on the rate of CO_2 uptake per dm^2 of leaf area. Note the considerable variation in rate of CO_2 uptake per dm^2 and frequent lack of correlation of these rates with total CO_2 assimilation at different leaf positions. The estimated total hourly CO_2 input per plant was predicted to be 493 and 1,600 mg CO_2 (rather than the observed 325 and 2,613 shown) for day 7 and day 20, respectively. Predicted values were obtained from multiple regression analysis of measured daily CO_2 input *versus* irradiance and time (see text and Table III).

Leaf Position	Rate of Photosynthesis		Avg. Photosynthetic Rate for Entire Leaf	
	Day 7	Day 20	Day 7	Day 20
No.	*mg $CO_2/dm^2 \cdot h$*		*mg CO_2/leaf·h*	
4	2.4 (1)	−3.0 (1)	12.6	−11.3
5	10.8 (1)	−3.6 (1)	69.1	−26.8
6	7.8 (2)	9.8 (2)	59.5	96.3
7	2.1 (1)	12.8 (2)	17.5	131.0
8	6.3 (2)	20.4 (2)	59.9	260.3
9	5.4 (2)	24.2 (2)	44.5	258.3
10	2.7 (1)	27.8 (1)	24.4	315.6
11	1.8 (3)	13.9 (3)	18.6	116.0
12		22.1 (1)		304.8
13		28.3 (3)		305.0
14		32.2 (2)		280.9
15		35.3 (1)		194.2
16		27.8 (2)		86.8
17		30.5 (2)		134.1
18		17.3 (4)		83.7
19		(0)		40.0[a]
20		19.9 (1)		44.1
Avg. rate	5.2 (SD = 3.7)	19.7 (SD = 11.5)		
Total hourly rate for entire plant			325.0	2,613.0

[a] Estimated value at this leaf position.

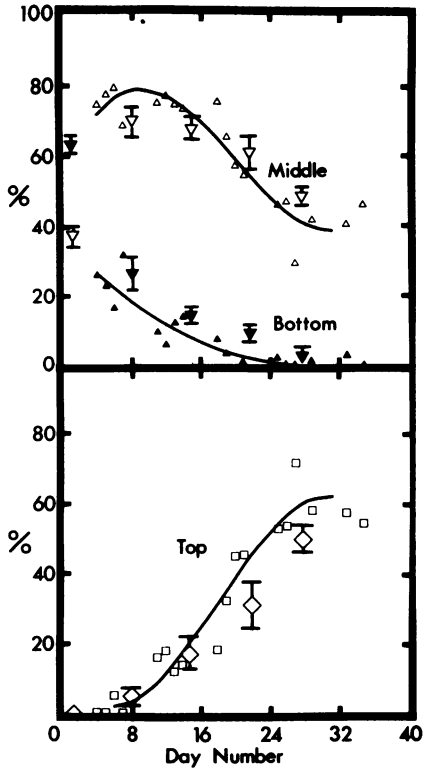

FIG. 5. Relationships between percent leaf area for variety 2 and relative contribution of CO_2 by photosynthesis for three strata within the plant canopy *versus* day number. Estimates of CO_2 input into the entire canopy were performed using the CO_2-depletion technique on 18 separate days for periods of about 8 h (see text). Relative CO_2 inputs into the bottom (leaf positions 3 to 5), middle (leaf positions 6 to 12), and top (leaf positions 13 to 20) are shown along with third-order regression lines (r^2 = 0.83, 0.90, 0.94 for bottom, middle, and top, respectively). Large symbols showing error bars (±1 SE, n = 5), show the proportion of total leaf area occurring within the respective zones. The CO_2 input is strongly correlated with leaf area in the middle and top zones. Little CO_2 input arose from the bottom zones because of heavy shading and aging of leaves. Similar results were observed for variety 1.

The leaf canopies of the test plants during the 35-d experiment could be divided into three zones on the basis of stage of leaf development (Fig. 5). Zone 1 included the bottom leaves (positions 3 to 5) which were fully expanded by day 1, and whose contribution to the total leaf area continually declined during the season due to progressive shedding. Leaves in zone 2 (middle; positions 6 to 12) had already emerged by day 1 and were present throughout the season. Leaves located on the top portion of the plant that emerged and expanded during the course of the study comprised zone 3 (positions 13 to 20). Most of the CO_2 fixation (greater than 90%) during the experiment occurred in zones 2 and 3. The relative contributions of each of zones 2 and 3 to the total leaf area of the plant over the course of the season fall close to the respective regression lines for relative contribution to total CO_2 fixation. The results indicate that respective leaf areas rather than respective rates of CO_2 input per dm^2 of leaf surface area in zones 2 and 3 largely determine the relative inputs of CO_2 into these zones. The data also suggest that mutual shading of leaves in zone 2 was similar to that occurring in zone 3. This undoubtedly results from the wide spacing between plants (1.8 m) which precluded formation of a continuous closed canopy over the plot. The small contribution to total CO_2 assimilation by leaves in zone 1 in comparison to relative leaf area in that zone resulted from severe shading by upper leaves and senescence of the lower leaves. Clearly, the relative importance to total CO_2 input of different strata within the canopy changes throughout the season.

The results in Table II for an overcast day and a sunny day reflect the uncertainty in attempting to relate crop growth rate to photosynthetic activity by taking measurements at just one or a few leaf positions. There is considerable variation in net CO_2 exchange per unit of leaf area arising from changes in total solar input from day to day and from mutual shading effects and differing leaf ages at the various leaf positions. Owing to the range of leaf areas encountered at the various leaf positions of a plant, no direct relationship is apparent between mg $CO_2/dm^2 \cdot h$ and total CO_2 assimilation per leaf per hour. Values of 325 and 2,613 mg CO_2/plant·h were obtained for an overcast and a sunny day, respectively, by summation of the average inputs at all leaf positions (see above). Predicted values for the same days, however, were estimated to be 493 and 1,600 mg CO_2/plant·h, respectively, based on a simple model relating daily whole plant CO_2 input to irradiance and total leaf area using measurements made on 18 different days during the 35-d period (see below).

Model for CO_2 Input into Whole Plants. Since it was impractical to sample CO_2 exchange during all hours of every day during the experiments, a simple model was prepared to relate measurements to predicted daily CO_2 inputs. Irradiance and total leaf area were assumed to be the two most important variables in predicting photosynthetic CO_2 input into the leaf canopy (*i.e.* mg CO_2/plant·h = $f(I)g(A)$ where I = irradiance and A = total leaf area). Rates of photosynthesis by single leaves or canopies are hyperbolically related to irradiance ($f(I) = I/(I + I_m)$ where I_m is the irradiance at which photosynthesis is half of maximal) (14). The value of I_m for the whole plant would be expected to increase with time from a minimal value of 10 to 15 cal/$cm^2 \cdot h$, observed with single leaves of tobacco (7, 14), as mutual shading of leaves progresses during leaf canopy development. A mean value for I_m was chosen for this model based on studies relating LAI[1] to CO_2 input. A closely spaced stand of tobacco will have LAI of about 8.5 (14). Since the plants in this study were spaced 1.8 m apart, we have assumed that the LAI has been lowered to about 4. Soybean plants with a LAI of 4 exhibit a I_m of about 35 cal/$cm^2 \cdot h$ (8). This value produced a good fit of observed whole plant rates of CO_2 input to the model based on multiple regression analysis (see below).

During relatively short intervals of growth, CO_2 input should be directly proportional to total leaf area. However, over the course of a season senescence may diminish the photosynthetic capacity of some of the leaves. The effect of total leaf area on CO_2 input has been assumed to increase with time (T) according to a third order polynomial ($g(T) = A_1 + A_2T + A_3T^2 + A_4T^3$ where A_{1-4} are constants).

The model of CO_2 input presented earlier may be expanded to include the respiration rate of the entire leaf canopy (S), *i.e.* mg CO_2/plant·h = $f(I)g(A) + S$. When I = 0, the net CO_2 exchange rate becomes negative representing leaf canopy CO_2 evolution. The value of S varied over the season primarily due to changing total leaf area per plant (see below). Table III shows individual multiple regression analyses, for varieties 1 and 2, of the observed rates of whole plant CO_2 input minus the respective rates of dark respiration by the leaf canopy *versus* irradiance and day number according to the model proposed above. The values for r^2 of 0.88 and 0.86 for varieties 1 and 2, respectively, suggest that the proposed model can reliably predict CO_2 input into the stands.

Predicted values of daily CO_2 input for each of the 35 d were obtained by substitution of day number and average irradiance (total solar input in cal/cm^2·15 h photoperiod) into the model. Respiratory CO_2 losses were subtracted (see below) to yield predictions of net CO_2 uptake and of dry weight accumulation during the 35-d experiment (Fig. 3).

[1] Abbreviation: LAI, leaf area index

Table III. *Multiple Regression Analyses of Whole Plant Photosynthetic Rates for Two Varieties of Field-Grown Tobacco*

Rates of photosynthesis were calculated by summation of the observed mean rates of CO_2 input at the individual leaf positions (see text). Irradiance was determined by dividing the total solar input during the sampling period by the number of hours in that period. Multiple regression analyses of rates of CO_2 input minus dark respiration by the leaf canopy (S) for each of the varieties were made against day number (T) and irradiance (I) by assuming that g CO_2/plant·h $= S + (I/I + I_m)(A_1 + A_2T + A_3T^2 + A_4T^3)$ where $I_m = 35$ cal/cm^2·h and A_{1-4} are constants (see text). Rates of dark respiration by the leaf canopy were estimated from the measured rates of dark respiration by leaves (Table V). Total leaf areas were obtained from the stem diameters of the test plants (see Fig. 2). Degrees of freedom associated with the regression and error were 4 and 13, respectively.

Day	Irradiance	Photosynthetic Rate		Leaf Canopy Respiration Rate	
		Variety 1	Variety 2	Variety 1	Variety 2
	cal/cm^2·h	*g CO_2/plant·h*			
4	51.3	1.22	0.99	−0.16	−0.14
5	35.9	1.33	0.85	−0.17	−0.15
6	48.4	0.96	0.79	−0.17	−0.14
7	22.0	0.60	0.33	−0.18	−0.17
11	66.7	1.24	1.56	−0.21	−0.19
12	48.3	1.42	1.49	−0.26	−0.23
13	28.5	0.87	0.77	−0.26	−0.20
14	51.6	2.21	1.32	−0.30	−0.18
18	63.2	2.49	1.65	−0.36	−0.22
19	46.9	1.82	1.87	−0.34	−0.33
20	68.4	2.20	2.61	−0.36	−0.37
21	46.5	2.45	2.48	−0.39	−0.42
25	47.1	2.38	2.39	−0.46	−0.47
26	53.9	2.33	1.65	−0.45	−0.46
27	69.0	2.69	2.28	−0.51	−0.52
29	45.8	2.08	2.25	−0.43	−0.48
33	48.7	2.34	2.23	−0.50	−0.50
35	62.7	2.08	2.37	−0.52	−0.51
Multiple regression analyses:					
R^2		0.88	0.86		
F		23.1[a]	20.3[a]		
SE of the estimate		0.30	0.35		

[a] $P < 0.001$.

Comparison of Seasonal Rates of mg CO_2/h·dm^2 of Total Leaf Area. Rates of whole plant CO_2 fixation for the 18 d during which sampling occurred were computed from the summation of inputs at all leaf positions and divided by the total leaf area (as approximated by the stem diameters of the test plants) as shown in Table IV. An analysis of variance of whole plant rates of CO_2 fixation per dm^2 of total leaf area for the 18 d indicated that no significant difference existed between the two varieties regarding this characteristic. These results together with those in Table I suggest that plants of equal total dry weight from the two varieties were nearly identical in total net photosynthetic capacity. It is evident that the consideration of total leaf area and rates of CO_2 fixation per unit of leaf area alone are not sufficient to determine total dry weight. Respiratory CO_2 losses in the dark must be considered, for example. Construction of a carbon budget enables an assessment to be made of various factors that determine final dry weight yield.

Respiratory CO_2 Losses. Photosynthetic CO_2 input was diminished by the amount of CO_2 lost by respiration in the leaves at night and in the roots and stems at all times during the 24-h day. Respiration rates of excised stems were related to diameters at the stem bases by a second order polynomial function (Fig. 6). No significant difference was discerned between the two varieties regarding stem respiration *versus* stem diameter. Stem respiration in the light was only 50% of that observed in the dark indicating occurrence of some refixation of respired CO_2 by the chlorophyllous epidermal cells. Daily CO_2 loss due to stem respiration was calculated on the basis of refixation of 50% of dark respiration during the 15-h photoperiod.

Leaf respiration differed to a small but statistically significant extent between the two varieties (Table V). Total nightly (9 h) CO_2 evolution from the leaf canopy was calculated by multiplying the total leaf area per plant by the mg CO_2 released/dm^2·h by respiration. Average values obtained after sunset on days 12, 18, and 25 were used in calculating leaf respiratory CO_2 loss for days 1 to 17, 18 to 24, and 25 to 35, respectively. Regression analyses of stem diameter *versus* day (not shown) were used in predicting values of total dry weight, total leaf area (Fig. 2), and stem respiration (Fig. 6) to be used in estimating daily respiratory CO_2 loss per plant.

A typical set of measurements used to estimate root respiration is shown in Figure 7. Rates of root respiration obtained by this method showed considerable variability and are considered to be only approximations. Mean rates of CO_2 evolution by root systems were 1.28 (SE = 0.35, $n = 6$) and 0.85 (SE = 0.13, $n = 7$) mg CO_2/g total plant dry weight·h for varieties 1 and 2, respectively. Estimates of mg CO_2/plant·d evolved by the root system were based on the mean total dry weight per plant as given by the stem diameter data (see above).

Total CO_2 inputs and estimated respiratory CO_2 losses for both varieties during the 35-d experiment are shown in Table VI. About 41% to 47% of the total CO_2 taken up was lost due to dark respiration in these varieties. Greater total CO_2 input by variety 1 during the experimental period is the result of its greater total leaf area and is consistent with its larger mean total dry weight compared to variety 2 (Figs. 3 and 4, Table I). Relative growth rates for varieties 1 and 2 appeared to be quite similar during the experimental period as were rates of CO_2 input per unit of total leaf area (Table IV). The varietal differences in dry weight yield

Table IV. *Mean Net CO_2 uptake per Hour per Unit of Total Leaf Area at Various Times during the Growing Season for Two Varieties of Field-Grown Tobacco*

The mg $CO_2/dm^2 \cdot h$ values shown are calculated according to the equation

$$\sum_{i=3}^{20} \overline{(S_i \cdot L_i)}/TLA$$

where S_i = measured mg $CO_2/dm^2 \cdot h$ at leaf position i, L_i = leaf area at leaf position i as predicted from the stem diameter (see text), $\overline{(S_i \cdot L_i)}$ = mean mg CO_2/leaf·h at position i during the sampling period of the day indicated, TLA = average total leaf area of the five test plants as predicted from their respective stem diameters (Fig. 2). Analysis of variance indicated that most of the variation in the values of mg $CO_2/dm^2 \cdot h$ was associated with days. The difference between the means of the two varieties was not significant at the P = 0.05 level. The SE was 0.45 (relative SE = 3.3%), and the least significant difference was 1.33.

Day	Irradiance	Photosynthetic CO_2 Uptake	
		Variety 1	Variety 2
	cal/cm²·h	*mg $CO_2/dm^2 \cdot h$*	
4	51.3	16.6	16.2
5	35.9	17.5	13.2
6	48.4	12.7	12.9
7	22.0	7.3	4.5
11	66.7	13.3	19.5
12	48.3	12.2	15.4
13	28.5	8.5	9.0
14	51.6	18.6	17.0
18	63.2	17.3	17.2
19	46.9	13.0	15.7
20	68.4	14.5	19.4
21	46.5	14.9	16.5
25	47.1	12.3	14.0
26	53.9	12.3	9.7
27	69.0	12.7	11.8
29	45.8	11.7	12.5
33	48.7	11.3	11.9
35	62.7	9.6	12.4
Seasonal mean		13.1	13.8

Analysis of variance:

Source	Degrees of Freedom	Mean Squares	*F*
Variety	1	4.34	1.20
Day	17	20.11	5.58[a]
Error	17	3.60	
Total	35		

[a] Significant at P = 0.001 level.

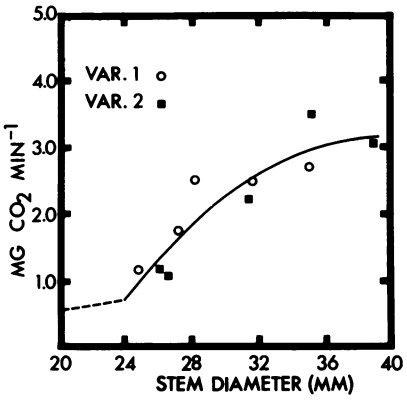

FIG. 6. Dark respiratory CO_2 release by tobacco stems *versus* stem diameter. Stems vere cut into 10-cm lengths and sealed in airtight steel cylinders (1 L volume) fitted with rubber septa. Incubations were for 10 min at ambient temperature (20–28°C). Air samples (5.0 ml) were withdrawn at the beginning and end of the sampling period. Successive assays of stems before and after cutting into smaller lengths suggested that cutting did not significantly alter the respiratory rate of the stem tissue. The line shown (——) is a second-order polynomial fit to the combined observations for varieties 1 and 2 obtained from regression analysis (r^2 = 0.85). The CO_2 evolution rates for stems for 24 mm or less in diameter were estimated assuming that these rates were directly proportional to stem diameter.

Table V. *Dark Respiration by Field-Grown Tobacco Leaves*

Two-min assays of CO_2 exchange (negative signs indicate CO_2 evolution) were conducted for the first two h after sunset on the days shown. The values shown are means of five to six measurements made on leaves at different positions on five plants. No consistent relationship was observed between leaf position and respiratory rate. Values in parentheses indicate range of values observed. The *F* values shown for the two-way analysis of variance are significant at the P = 0.05 level.

Day	Mean Respiratory Rate	
	Variety 1	Variety 2
	mg $CO_2/dm^2 \cdot h$	
12	−2.2 (−1.6 to −3.4)	−2.4 (0 to −5.3)
18	−2.5 (−1.3 to −3.7)	−2.8 (−2.0 to −3.5)
25	−2.4 (−1.1 to −4.5)	−2.7 (−1.9 to −3.7)

Analysis of variance:

Source	Degrees of Freedom	*F*
Day	2	39.00
Variety	1	64.00
Error	2	

observed during the experiment probably arose from differences in rates of seedling growth or adaptability to the field environment which occurred before the measurements began (Fig. 3).

DISCUSSION

It is often stated that there is a 'paradox' of no correlation between rates of CO_2 exchange per unit of leaf area and crop yield (6). However, this conclusion is frequently based on a comparison of instantaneous measurements of CO_2 exchange conducted under standardized conditions rather than on seasonal measurements. In fact, a few studies demonstrate that net CO_2 exchange on a seasonal basis is closely related to dry weight accumulation in the field (18).

Previous attempts to approximate a carbon budget in the field relied on portable assimilation chambers (4, 9, 11, 15). Occasionally, micrometeorological measurements of the gradients of CO_2 above the canopy were made to estimate the rate of flux of CO_2 from the atmosphere into the crop (3). The use of portable chambers required a cumbersome system for pumping air into and out of the chamber through flexible tubing and monitoring the CO_2 decrease, or the addition of measured quantities of CO_2 to maintain a constant CO_2 level. The time for such measurements varied from 2 to 15 min or longer, and frequently temperature control systems were needed as well as a means of transporting the chamber from one replicate plot to the next. Such methods can be quite complex and expensive. The CO_2-depletion method (5, 13) using hand-held chambers is easily portable, and since the measurement is completed in about 20 s (Figure 1), the temperature is essentially the same inside the chamber as outside. We are

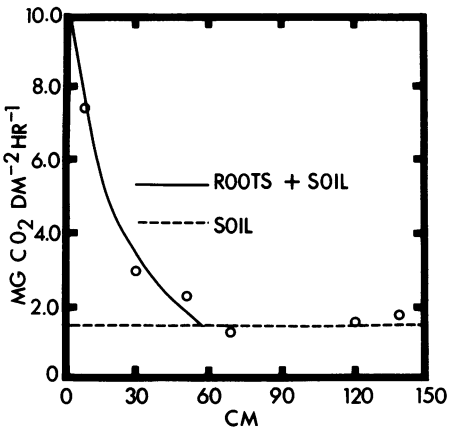

FIG. 7. An example of CO_2 evolution from the soil *versus* distance from the stem of a field-grown tobacco plant used to estimate root respiration. Steel cylinders (diameter = 10 cm) were positioned with their distal edges at the distances shown from the stem of the test plant. The edges of the cylinders were driven about 3 cm into the soil to prevent leakage of captured CO_2. The area enclosed was 0.79 dm^2 and the headspace volume was 0.79 L. Zero time gas samples were withdrawn from the cylinders and another set of samples were taken after 10 min. The CO_2 concentrations of the samples were determined as described in "Materials and Methods." Rates of soil CO_2 evolution were highest near the base of the plant and declined exponentially with distance to about 60 cm. The lower and reasonably constant rate of CO_2 evolution beyond 60 cm from the stem represented background soil respiration arising from microorganisms and invertebrates. Thus, the area enclosed by the curves for soil and roots-plus-soil represented root respiration. Total respiration by the root system was estimated by appropriate integration of CO_2 evolution over distance from the base of the plant.

Table VI. *Carbon Budget of Field-Grown Tobacco during a 35-d Experiment*

The dry wt increases for each variety during the 35-d sampling period were calculated from the regression lines in Figure 3. Values in parentheses are percent of total CO_2 input. See text for further details.

	Variety 1, CO_2 Equivalents	Variety 2, CO_2 Equivalents
	g	
Total CO_2 input	749.1 (100)	645.9 (100)
Respiration losses		
Leaves (night)	−102.3 (13.7)	−98.2 (15.2)
Roots	−186.7 (24.9)	−103.1 (16.0)
Stems	−64.0 (8.5)	−63.0 (9.8)
Total	−353.0 (47.1)	−264.3 (40.9)
Net input from CO_2 exchange measurements	396.1 (52.9)	381.6 (59.1)
Observed dry wt increase (CO_2 equivalents)	409.5	357.5

thus able to carry out accurate CO_2 exchange measurements on 30 leaves on each of two varieties (540 measurements per variety over the season) during an 8-h day and have the CO_2 exchange rates/dm^2·h available the same day. These instantaneous measurements could be integrated into a model of CO_2 input over the season which provided reasonable predictions of dry weight accumulation, thereby confirming the relationship between net CO_2 exchange rate and dry weight accumulation in the field (Table VI).

It is not possible to estimate photosynthetic CO_2 assimilation in the field from a few instantaneous measurements, as illustrated in Table II. Considerable variation is evident among individual leaf positions in the stand on any given day. Analysis of CO_2 input is facilitated by dividing the leaf canopy into groups composed of leaf positions at similar stages of development. As would be expected, this revealed that the pattern of relative CO_2 input by the respective groups can change dramatically over the season (Fig. 5). Hence, it is unlikely that a valid estimate of CO_2 input into the entire crop can be made from measurements at one or a few leaf positions without prior information about the relationship between stage of development and CO_2 input. This applies no matter how numerous or reliable the accumulated measurements are. Even as many as a total of 30 measurements performed at all leaf positions represented among five test plants of a variety during 1 d would likely yield an estimate of photosynthesis by the stand that by itself is unreliable (Table II). However, a large number of measurements made over the course of a season on all of the plants in a stand can provide a more sound statistical basis for comparison of photosynthetic characteristics between varieties (Tables III and VI). How many measurements might be needed and the relative contribution of various errors to estimates of daily CO_2 input per plant are discussed below.

A major purpose of this investigation was to determine whether the method employed would be sufficiently sensitive to permit recognition in future varieties of increased rates of CO_2 exchange. Decreasing the rate of photorespiration in C_3 leaves by genetic changes should increase the rate of CO_2 exchange per unit leaf area compared to otherwise isogenic cultivars possessing normal photorespiration. Based on the losses caused by photorespiration, increases of CO_2 exchange rate of as much as 50% may be possible (17). Identifiable sources of variation in estimating CO_2 exchange in a single leaf position were (a) error inherent in the CO_2-depletion method of assay, (b) error associated with estimates of leaf area, and (c) variation in mg CO_2/dm^2·h due to varying irradiance and mutual shading. Repetitive determinations (n = 10) on fully illuminated leaves in the field showed that the coefficient of variation of the CO_2 uptake assay was about 6.5%. More uncertainty was associated with the estimation of leaf area at different leaf positions based on the stem diameter of the plant (coefficient of variation of about 29%). Uncertainty in estimating leaf CO_2 input arising from variable irradiance [(c) above] was judged to far outweigh that associated with the other sources. Coefficients of variation approaching 100% were observed for some of the estimates of mg CO_2/h at a given leaf position based on measurements made on different test plants during 1 d. Thus, the coefficient of variation would probably be less than 26% for an estimate of whole plant mg CO_2/h from combined input by 15 leaves. Coefficients of variation of 14% and 18% for varieties 1 and 2 were associated with predictions of daily CO_2 input based on the model shown in Table III.

A comparison of whole plant mg CO_2/dm^2·h values and their means (Table IV) showed that most of the observed variation arose from the differences in daily solar input and the gradual decrease in rate as the season progressed. The whole plant mean values of mg CO_2/dm^2·h were not significantly different from each other, and it is unlikely that additional sampling of leaves would have established a significant difference between the two varieties. However, an observed difference of as little as 10% would have been significant at the P = 0.05 level in this investigation. Assuming that the coefficient of variation due to experimental error (3.3%) derived from Table IV is a good approximation of coefficients of variation due to error that might be expected for other tobacco varieties in future field experiments, cultivars possessing superior net photosynthetic rates (mg CO_2/dm^2·h) because of decreased photorespiration should be easily identifiable in the field. Moreover, a correspondingly larger difference in mean

whole plant mg $CO_2/dm^2 \cdot h$ of about 20% could be recognized as significant with as few as 4 d of sampling of the kind described here.

The estimated total CO_2 uptake per plant was greater for variety 1 than variety 2 (Fig. 3; Table VI), even though overall mean rates of CO_2 exchange/$dm^2 \cdot h$ for the two varieties were not significantly different when averaged over the whole season (Table IV). Thus, the greater CO_2 assimilation and dry matter accumulation in variety 1 was largely the result of a greater leaf area (Table I; Figs. 3 and 4). These characteristics were probably fixed at the seedling stage before the measurements of growth and CO_2 assimilation were begun and continued throughout the season (Fig. 3; Table I).

The CO_2-depletion method described offers a simple, direct, and inexpensive means of quantifying photosynthesis in the field. The method should be immediately adaptable to the study of CO_2 exchange by a number of important crop plants, and should be of special value in recognizing superior photosynthetic rates by leaf canopies.

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LITERATURE CITED

1. BERLYN MB 1978 A mutational approach to the study of photorespiration. *In* HW Siegelman, G Hind, eds, Photosynthetic Carbon Assimilation. Plenum, New York, pp 153–164
2. BERLYN MB, I ZELITCH, PD BEAUDETTE 1978 Photosynthetic characteristics of photoautotrophically grown tobacco callus cells. Plant Physiol 61: 606–610
3. BISCOE PV, RK SCOTT, JL MONTEITH 1975 Barley and its environment. III. Carbon budget of the stand. J Appl Ecol 12: 269–293
4. CHRISTY AL, CA PORTER 1981 Canopy photosynthesis and yield in soybean. *In* Govindjee, ed, Photosynthesis—Applications to Food and Agriculture. Academic Press, New York, In press
5. CLEGG MD, CY SULLIVAN, JD EASTIN 1978 A sensitive technique for the rapid measurement of carbon dioxide concentrations. Plant Physiol 62: 924–926
6. ELMORE, CD 1980 The paradox of no correlation between leaf photosynthetic rates and crop yields. *In* JD Hesketh, JW Jones, eds, Predicting Photosynthesis for Ecosystem Models. Vol II. CRC Press, Boca Raton, pp 155–167
7. HESKETH JD, DN MOSS 1963 Variation in the response of photosynthesis to light. Crop Sci 3: 107–110
8. JEFFERS DL, RM SHIBLES 1969 Some effects of leaf area, solar radiation, air temperature, and variety on net photosynthesis in field-grown soybeans. Crop Sci 9: 762–764
9. KANEMASU ET, CK HIEBSCH 1975 Net carbon exchange of wheat, sorghum, and soybean. Can J Bot 53: 382–389
10. PETERSON RB 1982 Stereochemistry of ^3H labeling in glycolate from tobacco leaf discs after photosynthesis in the presence of ^3H_2O. Plant Physiol 69: 192–197
11. PUCKRIDGE DW 1971 Photosynthesis of wheat under field conditions. III. Seasonal trends in carbon dioxide uptake of crop communities. Aust J Agric Res 22: 1–9
12. SPLINTER WE, JF BEEMAN 1968 The relationship between plant stem diameter and total leaf area for certain plants exhibiting apical dominance. Tobacco Sci 12: 139–143
13. SULLIVAN CY, MD CLEGG, JM BENNETT 1976 A new portable method for measuring photosynthesis. Agron Abstr, p 77
14. TURNER NC, LD INCOLL 1971 The vertical distribution of photosynthesis in crops of tobacco and sorghum. J Appl Ecol 8: 581–591
15. VIETOR DM, RB MUSGRAVE 1979 Photosynthetic selection of *Zea mays* L. II. The relationship between CO_2 exchange and dry matter accumulation of two hybrids. Crop Sci 19: 70–75
16. ZELITCH I 1979 Photosynthesis and plant productivity. Chem Eng News 57: 28–48
17. ZELITCH I 1979 Photorespiration: studies with whole tissues. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology, New Series, Vol 6, Photosynthesis II. Springer-Verlag, Berlin, pp 353–367
18. ZELITCH I 1982 The close relationship between net photosynthesis and crop yield and the critical role of oxygen stress. BioScience. In press