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Communication

Whole Plant CO_2 Exchange Measurements for Nondestructive Estimation of Growth[1]

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ABSTRACT

A computer controlled semiclosed net CO_2 exchange measurement system, employing an infrared gas analyzer and mass flow controllers to inject pure CO_2 at preset rates, has been developed for measuring whole plant net CO_2 exchange and net C gain in a controlled environment (*i.e.* CO_2, light, and temperature). Data for tomato (*Lycoperscicon esculentum* cv Campbell 19 VF) and rose (*Rosa hybrida* cv Samantha) plants grown for 4 and 17 day periods, respectively, clearly show that net C gain measured and computed using nondestructive CO_2 analysis equaled the increase in C content determined by chemical analysis following destruction of the test plants. The analysis of C gain based on CO_2 exchange allows estimation of biomass production and growth of a single population of plants under varying light and CO_2 conditions without physically handling the test plants.

Many gas-exchange systems based on infrared gas analysis of CO_2 have been designed to measure photosynthesis, photorespiration, and dark respiration of single plant leaves (2, 9, 15). However, measurement of leaf photosynthesis does not necessarily predict plant growth and crop productivity (3), since individual leaves are not representative of the photosynthetic behavior of the entire canopy. Furthermore, analysis of the metabolism of a single leaf does not take into account dark respiration of the entire plant and ignores both the problem of partitioning of photoassimilates and the evaluation of crop quality (5, 14). A positive correlation between leaf photosynthetic rate and crop productivity requires considerable sampling (3, 12) and is most easily obtained when studying a crop for which the vegetative portion of the plant (*e.g.* leaves or roots) is harvested for market (8, 14, 17). In spite of the problems in correlating photosynthesis with yield it is well known that over 95% of the dry matter of a plant is derived from photosynthesis and further that carbon (C) obtained from photosynthesis comprises approximately 40% of the plant dry weight under most growth conditions (1, 7). Even so, CO_2 analysis itself is rarely used as a means of measuring growth rate.

Growth rate is frequently defined as an increase in the physical size of the plants expressed simply as an absolute increase in dry weight with time (*e.g.* g gained · week^{-1}) or as a relative increase in size (*e.g.* g gained · week^{-1} g initial weight of the plant). Unfortunately, the most accurate method of obtaining the actual dry weight is to sacrifice individual test plants, a protocol which makes it impossible to obtain the IDW[3] of individual test plants. Furthermore, many parallel samples must be grown so that they can be sacrificed at specific times during the experiment. The variation between plants is often minimized by selecting uniform plant material from a large population and estimating the IDW by subsampling this population (4). Analysis of net C gain (ΔC) achieved by monitoring net CO_2 exchange rate nondestructively seems to be an alternative procedure to destructive dry matter analysis particularly in studies of absolute growth rate. To obtain relative growth rate as well as absolute growth rate an estimate of initial size of the test plant or population is clearly required. In this regard a correlation coefficient between dry weight and some other measure of plant size (*e.g.* fresh weight) can be established and used to estimate IDW nondestructively.

Bate and Canvin (1) described a gas exchange system for studying the relationship between whole plant net photosynthesis and the productivity (biomass gain) of a small population of sunflower and aspen plants maintained in controlled environmental chambers. Although they did not control CO_2 levels in their chambers they showed that a CO_2 analysis system can be used to estimate the net C gain of a small population of plants with over 85% accuracy, provided sufficient care is taken to avoid errors in both the gas monitoring system and the determination of dry weight. Because of a long standing interest in CO_2 enrichment in horticultural science (14), many other workers have used controlled environment chambers connected to gas analysers to regulate CO_2 levels and intermittently measure whole plant gas exchange (6, 11). However, these studies do not include any attempt to correlate C gain measured by infrared gas analysis with plant growth.

The computer controlled system we outline here employs M as well as an IRGA to monitor and control chamber CO_2 levels. The data from the two instruments are used to calculate NCER. These values provide an accurate nondestructive method of estimating net C gain and therefore the growth rate of plants being maintained in a specified environment.

MATERIALS AND METHODS

Plant Materials. The reliability and usefulness of whole plant CO_2 gas analysis for estimating plant growth nondestructively is shown by the data obtained in three experiments outlined below. In the first two experiments a sufficient number of rose cuttings

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[3] Abbreviations: IDW, initial dry weight; ΔDW, net dry weight gain; ΔC, net C gain; M, massflow controller; IRGA, infrared gas analyzer; NCER, net carbon exchange rate; ΔCO_2, cumulative CO_2 uptake; IFW, initial fresh weight; FDW, final dry weight.

(*Rosa hybrida* cv Samantha) and tomato transplants (*Lycopersicon esculentum* cv Campbell 19 VF) were grown to measure the initial and final C content and dry weight of the test plants by conventional destructive analysis (4). The estimates of growth derived from these data were compared with estimates of carbon measured solely by nondestructive CO_2 analysis (NCER). In the third experiment reported below rose cuttings were maintained under two different environments (*i.e.* ambient and enriched CO_2 levels). Growth (C gain) was estimated solely by nondestructive CO_2 analysis (Fig. 2d).

In the case of the herbaceous crop, the tomato transplant population was very uniform. The IDW was estimated by sacrificing 20 plantlets and assuming an average plant weight of 0.86 g (Table I). A further 15 transplants, each growing in PRO-MIX BX (Premier Brands Inc.) in a 13.5 cm pot were placed in the three experimental chambers (5 plants per chamber) described below (Fig. 1). The transplants were maintained in a constant environment (25 ± 0.2°C temperature, 300 μmol m^{-2} s^{-1} light (PAR 400–700 nm), and 800 ± 20 μl L^{-1} CO_2) for 4 d during which time C gain was computed from CO_2 exchange data provided by the IRGA (model SS300, Analytical Development Company Ltd., Hoddesdon, England) and the massflow controller (model FC260, Tylan Corp., Carson, CA) as outlined below. At the end of the experiment all 15 test plants were harvested for determination of FDW and C content by chemical means (Table I).

In a second similar experiment, 15 rooted rose cuttings (3–5 cm long shoots) were grown in the analysis chambers for 17 d in a constant environment (22 ± 0.2°C temperature, 200 μmol m^{-2} s^{-1} light (PAR 400–700 nm) and 600 ± 20 μl L^{-1} CO_2). Rooted rose cuttings, although similar in initial size to each other, by no means represent as uniform a population of test plants as do the tomato seedlings. To estimate the IDW of the rose cuttings actually used in the growth experiments, the correlation between IDW and various parameters which could be determined in a nondestructive manner including leaf area, stem diameter, stem length, and IFW were compared. Of these parameters, IFW was best correlated to IDW with a correlation coefficient of 0.93. Thus, IFW was used to estimate IDW of the 15 rose plants actually used in the experiment (Table I).

Maintenance and Measurement of CO_2 in Controlled Environmental Chambers. A whole plant net CO_2 exchange system employing three specially constructed clear acrylic plant chambers was assembled in a conventional greenhouse. Each plant chamber (Fig. 1) measured 82 cm in height, 52 cm in length, and 45 cm in width. When in the open mode (*i.e.* to the outside atmosphere) fresh air was drawn into the plant chamber through V_1 at a flow rate of 30 L min^{-1} and exited the chamber via V_2. A circulating fan (Fig. 1) facilitated air movement over the plants while air temperature in the chamber was measured and controlled at a preset level (±0.2°C) with a YSI 44202 linear thermistor, T (Yellow Springs Instrument Co.) A preset CO_2 concentration (±30 μl L^{-1} CO_2) between 300 and 4000 μl L^{-1} can be maintained in each chamber by adding pure CO_2 to the air intake line before V_1 via a massflow controller, M_1. While the system was in the open mode (air entering and exiting the chamber via V_1 and V_2) the CO_2 level in the chamber was checked by drawing chamber air through V_3 to the instruments before returning it to the plant chamber via V_4 at a flow rate of 4 L min^{-1}. Air in the instrument sampling line was split between V_3 and V_4 so that flow through the humidity transmitter, HT (Dewtrack model 22, EG & G Environmental Equipment) and the IRGA were 3.5 and 0.5 L min^{-1}, respectively. When the CO_2 level of the chamber air was not at the preset level (*e.g.* 350 μl L^{-1} CO_2) the computer readjusted the flow of pure CO_2 through M_1. However, continuous daily recording (data not shown) indicated that in the open mode changes in the CO_2 level in the system due to plant metabolism (photosynthesis and/or respiration) were not frequently observed, presumably, because there was a relatively large volume of fresh air passing through the open system. The plant growth chamber remained in the open mode for over 90% of the experimental period.

Estimation of Net Carbon Exchange Rate and C Gain. In order to measure the NCER of the plants while chamber air passes through V_3 and V_4, V_1 and V_2 were closed effectively sealing the plants and the instruments (IRGA and M_2) from the outside (greenhouse) atmosphere. With the system closed, CO_2 level changed more dramatically due to plant metabolic activity.

When V_1 and V_2 were closed and the plants were illuminated, the CO_2 level dropped due to photosynthesis. In order to maintain a preset CO_2 level in the closed chamber, a second mass flow controller (M_2) was used to inject pure CO_2. The rate of CO_2 injection depended on the rate of depletion of CO_2 in the chamber. The amount of CO_2 injected to maintain the CO_2 level

Table I. *Estimation of Growth of Tomato and Rose Plants using Destructive and Nondestructive Analysis*

Tomato and rose plants were grown in controlled environment chambers for 4 and 17 d, respectively, as outlined in "Materials and Methods." The IDW of the five tomato transplants in each chamber was estimated from the average dry weight (0.86 g/plant) of 20 similar seedling samples. The IDW of the rose cuttings in each chamber was estimated from their IFW. The growth expressed as ΔDW was calculated as the difference between IDW and FDW. ΔC′ was estimated from ΔDW and the average carbon content of the harvested plants. Chemical analysis showed that the carbon content of tomato and roses were 35.4 and 39.7%, respectively. Growth was also determined nondestructively. The ΔCO_2 for each chamber was calculated as in "Materials and Methods" (Eq. 2). ΔC was calculated from ΔCO_2 (*i.e.* $\Delta CO_2 \times 12/44$). An indication of the accuracy of the nondestructive estimation of growth is given by the ratio of net carbon gain calculated by the two procedures (*i.e.* ΔC/ΔC′).

	Chamber	Destructive Growth Analysis				Nondestructive CO_2 Analysis		$\frac{\Delta C}{\Delta C'}$
		IDW	FDW	ΔDW	ΔC′	ΔCO_2	ΔC	
			g					*ratio*
Tomato	1	4.30	15.11	10.81	3.83	13.02	3.55	0.93
	2	4.30	15.14	10.84	3.84	13.95	3.80	0.99
	3	4.30	14.77	10.47	3.71	14.08	3.84	1.04
Rose	1	9.23	22.30	13.07	5.19	17.45	4.76	0.92
	2	6.92	14.71	7.79	3.09	12.09	3.30	1.07
	3	7.16	16.56	9.40	3.73	14.34	3.91	1.04

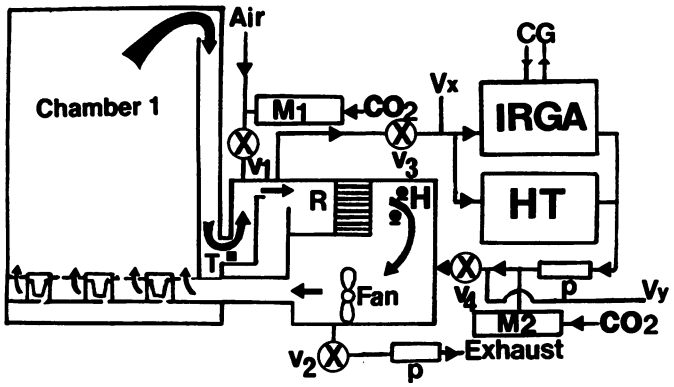

FIG. 1. Diagram of computer controlled growth chamber for measuring NCER and nondestructive estimating plant growth. M, massflow controller; IRGA, infrared gas analyzer; T, linear thermistor; V, electric solenoid valve; HT, humidity transmitter; CG, calibration gas; P, air pump; R, cooling radiator; H, resistive heater.

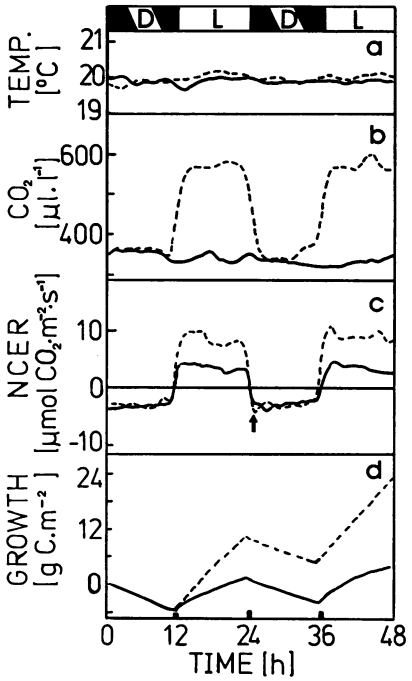

FIG. 2. Nondestructive CO_2 analysis for estimation of growth of rose plants grown at ambient and CO_2 enriched environments. Two growth chambers with three plants per chamber were used for this study. Each chamber temperature was maintained at 20°C (a). The CO_2 level in one chamber was maintained at about 350 µl L^{-1} (solid line in (b)) throughout the 12 h photoperiod (450 µmol m^{-2} s^{-1} PAR, 400–700 nm). In the other chamber, the CO_2 level was raised from 350 µl L^{-1} during dark (D) period to about 600 µl L^{-1} in the light (L) period (dashed line in (b)). NCER (c) based on CO_2 gas analysis was calculated using Eq. 1. Plant growth (d) expressed as carbon gain per leaf area (g C·m^{-2}) over the 48 h period was calculated from NCER using Eq. 2 as outlined in "Materials and Methods."

in the closed system was recorded along with IRGA readings. Readings from the IRGA for min 1 were not used in the calculation of NCER since a brief period was required for equilibration of air sampling lines and the chamber. Root respiration was included in the net CO_2 exchange measurement. Since plants were grown in soiless medium, microorganism respiration was considered insignificant so that CO_2 loss in the dark was used to determine whole plant dark respiration (Fig. 2c). The NCER of the plants was calculated from both IRGA and M_2 measurements as shown in the following equation:

$$\text{NCER} = \frac{\text{Vol} \times (C_2 - C_1)}{[0.0821 \times T \times (t_2 - t_1)]} + \frac{\text{Total } CO_2 \text{ injected}}{t_2 - t_1} \quad (1)$$

where Vol = chamber volume (L); C_1 = CO_2 concentration (µl L^{-1}) at t_1; C_2 = CO_2 concentration at t_2; 0.0821 = gas constant (1 $K^{\circ -1}$ mol^{-1}); T = absolute temperature (K°); t_1 = time 1 (s); t_2 = time 2.

After sufficient data were collected from the IRGA and M_2 by the computer the chamber was returned to the open mode by simultaneously turning off M_2 and opening V_1 and V_2. In the open mode the CO_2 levels in the chamber were maintained by injection of pure CO_2 through M_1 only. This operation usually required about 2 min. After a final check of the CO_2 level in the open chamber as described above, V_3 and V_4 were closed freeing the IRGA and M_2 for analysis of CO_2 drawn from the other plant chambers. Our experience is that in total one IRGA, and one massflow controller. M_2, feeding data to one computer can be used to measure and maintain CO_2 levels in four growth chambers of the size described above each equipped with its own mass flowmeter (M_1). The purpose of a M_1, associated with each chamber is to inject CO_2 when readings are not actually being recorded for CO_2 uptake analysis. Valves corresponding to V_3 and V_4 would be required for each additional chamber (Fig. 1, V_x and V_y). In order to study individual plants or larger populations more chambers employing another IRGA could be used.

Net carbon gain (Table I; Fig. 2d) was estimated nondestructively from the ΔCO_2 given by Eq. 2. These estimates of growth were compared to estimates obtained from the plants harvested and killed for chemical determination of C and dry weight (Table I).

$$\Delta CO_2 = \sum_{i=1}^{n} \left[\frac{(\text{NCER}_i + \text{NCER}_{i+1})}{2} \times (t_{i+1} - t_i) \right] \quad (2)$$

where i is any NCER measurement from 1 to n; n is the total number of NCER measurements during the experimental period; t_i is the time of NCER_i estimation; $t_{i+1} - t_i$ is the time interval between two consecutive NCER measurements (about 15–20 min) which corresponds to the period required for measuring CO_2 exchange among all the chambers using the IRGA and massflow controller system as outlined above.

Chemical Analysis of Tissue C. Total plant C was determined chemically using a modified dichromate wet digestion method outlined by Sims and Haby (16). Fifty mg of ground dry tissue was placed in 250 ml Erlenmeyer flask in 10 ml distilled water. Ten ml of 1 N $K_2Cr_2O_7$ and 20 ml of H_2SO_4 (96%, reagent grade) were added to each flask which was then capped with aluminum foil and heated for 2 h at 120°C. After cooling, the absorbance of the digested samples was measured at 600 nm with a DU-8 spectrophotometer (Beckman). Absorbance was linearly related to carbon content in the range of 0 to 60 mg sucrose (0–25.2 mg C equivalents).

RESULTS AND DISCUSSION

The results in Table I clearly show that net C gain determined by conventional chemical analysis for both roses and tomatoes was in close agreement with estimates of C gain measured using gas analysis. Net C gain accounts for 39.7 and 35.4% of the dry weight increase for rose and tomato plants, respectively. Ho (7) studied the variation in the C/dry weight (w/w) ratio in tomato and concluded that the C/dry weight ratio varies according to species, organ, and developmental stage as well as growing con-

ditions. Our results (data not shown) indicated that C content ranged from 36 to 43% for roses and 32 to 38% for tomato plants, depending on the tissue. In both crops, leaf tissue had higher C content than that of stem and root tissues. Although analysis of partitioning of C in the plant requires destruction of the test plant, the data obtained from nondestructive whole plant net CO_2 exchange measurements using our system can be used to accurately monitor plant growth (Table I; Fig. 2, c and d).

The results outlined in Figure 2 clearly show the value of a nondestructive method of assessing growth rates of a small number of plants under two commonly varied experimental conditions (*i.e.* light versus darkness and ambient versus CO_2 enriched conditions). Three rose plants with unequal initial dry weights (Table I) were placed in each of the two controlled-environmental chambers. All plants were subjected to 12 h light and darkness and maintained at the same temperature (20 ± 0.2°C, Fig. 2a). One set of plants was treated with 600 μl L^{-1} CO_2 during the light period (Fig. 2b) while the other set was maintained at 350 μl L^{-1} CO_2. In the light NCER calculated on leaf area basis was higher in the CO_2 enriched plants reflecting a greater photosynthetic rate (Fig. 2c). Interestingly, in the CO_2 enriched plants, dark respiration of the whole plants was initially greater following a period in the light than in the ambient grown plants which had clearly assimilated less C (arrow, Fig. 2c). However, the average total loss of C due to dark respiration of the CO_2 enriched plants over the first 12 h dark period was similar to that of plants grown under the ambient CO_2 condition during the previous light period (Fig. 2c). Peterson and Zelitch (12) noted that in tobacco dark respiratory losses in the field (*i.e.* ambient CO_2) accounted for 42 to 47% of the total CO_2 assimilated. Our data (Fig. 2d) show that at ambient CO_2 about 50% of the C assimilated during the day was lost during the following night period. By comparison, only 25% of the total C assimilated under CO_2 enriched condition was actually lost due to dark respiration during the following night consistent with the retention of more photoassimilate, initially in the form of leaf starch (10). After several days the CO_2 enriched plants partition more C into new leaves establishing a greater leaf area which sustains a greater whole plant photosynthetic rate (10) even though the photosynthetic efficiency of individual mature leaves may begin to decline (13).

In summary, the system described here provides us with the ability to examine net CO_2 exchange of whole plants and nondestructively correlate physiological events with growth. A nondestructive growth analysis system which both monitors gas exchange and controls the plant growth environment may also be of practical importance in a space research program where weight analysis may be impractical.

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