

## CO<sub>2</sub> response curves can be measured with a field-portable closed-loop photosynthesis system

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### Introduction

Assimilation rate *versus* internal CO<sub>2</sub> response curves provide an important tool for assessing the efficiency and capacity of the photosynthetic system. Until recently, measurement of CO<sub>2</sub> response curves was limited to laboratory studies, where elaborate gas exchange systems were available, or to mobile field laboratories. Here we report the use of a portable photosynthesis system (LI-6200, LI-COR, Inc.) for measurement of response curves. The LI-6200 uses a closed-loop design in which varying CO<sub>2</sub> concentrations are provided as the leaf removes CO<sub>2</sub> from the system. A typical measurement requires 10–25 min, depending upon chamber volume, leaf area and assimilation rate. Response curves measured on well-watered soybean and cotton with the LI-6200 are compared to those measured

with a fully controlled steady state system. The effects of system leaks and control of leaf temperature are discussed.

### Materials and Methods

Data of Fig. 1 were obtained on well-watered soybeans (*Glycine max* (L.) Merrill, cv *Hobbit*) grown in soil and 12 in pots in a temperature-controlled (27 ± 3°C) greenhouse in Lincoln, NE. Measurements were made on upper canopy fully exposed leaves when the plants were in the early pod-filling stage. PAR was supplied by one Metalarc 400 W lamp and one Lucolux 400 W lamp in a single water-cooled luminaire (Sunbrella, Environmental Growth Chambers, Chagrin Falls, OH). The 1 l chamber of the LI-6200 was mounted on a tripod and placed at a distance beneath the lamp which gave the desired light intensity. Radiation from the lamp was filtered with 1/4 in plexiglas and external air flow was provided by a small 110 V fan. Response curves were constructed as described in results.

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Data of Figs. 2, 3 and 4 were obtained on vegetative soybeans grown in vermiculite and 8 in pots in the greenhouse at Carnegie Institution, Stanford, CA. Measurements were made in an adjacent laboratory with the steady state system described by Ball (1987), and with the LI-6200. Relative humidity sensor and IRGA calibrations were carefully compared and checked prior to measurement. PAR ( $1200\text{--}1300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) was supplied by a high intensity projector lamp filtered with a dichroic mirror. Comparative measurements were made on the same leaflets. Data reported in Figs. 2, 3 and 4 were obtained with chamber relative humidity (RH) above 72% in both systems. A response curve measured on soybean with the LI-6200 at ambient humidity (32%) deviated from a concomitant curve measured with the steady state system at about 70% RH. The pattern of photosynthesis rates and internal  $\text{CO}_2$  concentrations suggested that stomatal conductance was not uniform across the leaf at the lower humidity (Terashima *et al.*, 1988; data not shown). Data of Fig. 5 were obtained on vegetative cotton grown in nutrient solution at  $33^\circ\text{C}$ , about 35% RH and  $600\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light intensity. Further details pertaining to the measurements are given in the text.

## Results

A baseline  $\text{CO}_2$  response curve was measured by placing a single soybean leaflet in the 1 l assimilation chamber of the LI-6200 and allowing the leaflet to remove  $\text{CO}_2$  until the compensation point was reached. Assimilation rate, conductance and internal  $\text{CO}_2$  concentration were computed every 5 ppm or so as the chamber  $\text{CO}_2$  mole fraction declined. This was repeated 2 more times and all curves were coincident (data not shown). A 4th curve was prepared in which the  $\text{CO}_2$  mole fraction was held constant ( $\pm 5\ \mu\text{mol}\cdot\text{mol}^{-1}$ ) for 5 min at 7 different levels using a  $\text{CO}_2$  injector. Assimilation, conductance and  $C_i$  were then measured in transient mode by allowing the  $\text{CO}_2$  mole fraction to decline a few ppm from each of the preset levels (Fig. 1). Since

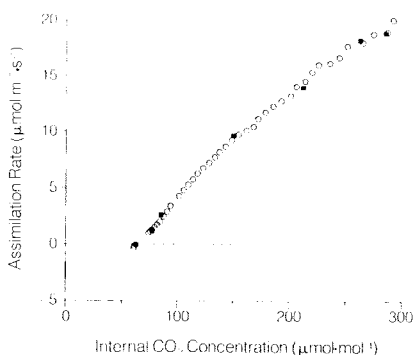
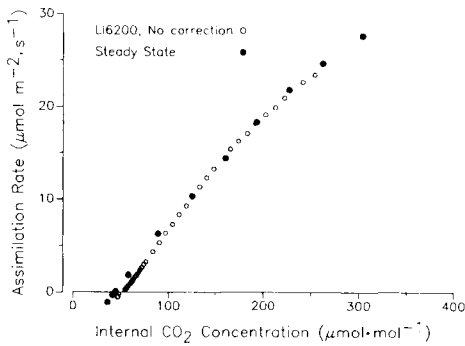


Fig. 1. A comparison of  $\text{CO}_2$  response curves measured in soybean during continuous drawdown (o) of chamber  $\text{CO}_2$  concentration, with a similar curve measured when chamber  $\text{CO}_2$  concentration was held constant ( $\pm 5\ \text{ppm}$ ) for 5 min before each measurement. Light intensity was  $1000 \pm 50\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Redrawn from Davis *et al.*, (1987)).

the curve measured by continuous drawdown is coincident with that measured after a 5 min equilibration at each  $\text{CO}_2$  level, we conclude that the 2 methods are equivalent. Soybean leaflets are evidently able to maintain a quasi-steady state with a slowly declining ( $0.01\text{--}1\ \text{ppm}\cdot\text{s}^{-1}$ ) external  $\text{CO}_2$  concentration. Three other experiments gave the same result.

To further evaluate results obtained with the LI-6200, response curves were measured on soybeans with a steady state system described by Ball (1987) and side-by-side measurements were made on the same leaves under similar conditions with the LI-6200 (Fig. 2). Correspondence between the 2 methods is generally excellent except that the  $\text{CO}_2$  compensation point is slightly overestimated by the LI-6200. At low chamber  $\text{CO}_2$  mole fractions, a large  $\text{CO}_2$  gradient exists between chamber air and ambient air exaggerating chamber leaks that are normally small. Leaks cause an underestimation of the assimilation rate, and consequently, an overestimation of the compensation point.



**Fig. 2.** A comparison of CO<sub>2</sub> response curves measured by continuous drawdown with the LI-6200, with curves measured on the same soybean leaflet using the steady state system. Conductances varied in the range 0.53–0.62 mol·m<sup>-2</sup>·s<sup>-1</sup> (LI-6200) and 0.42–0.54 (steady state system). Average leaf temperatures were 27.4°C (LI-6200) and 28.3°C (steady state). Leaf temperature variation was below 0.5°C in both systems.

Chamber leaks can be modeled by the following expression:

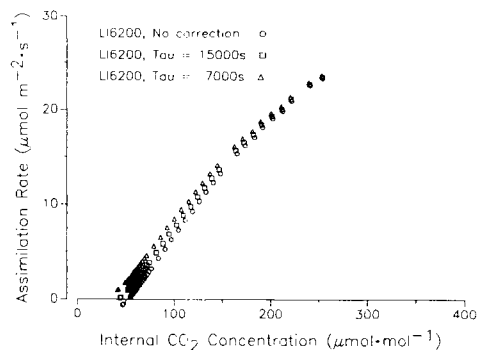
$$(dC_{\text{chamber}}/dt) = (C_{\text{ambient}} - C_{\text{chamber}}/\tau);$$

where  $dC_{\text{chamber}}/dt$  is the CO<sub>2</sub> change rate due to chamber leaks (s<sup>-1</sup>),  $C_{\text{ambient}}$  is the CO<sub>2</sub> mole fraction of ambient air surrounding the chamber (μmol·mol<sup>-1</sup> or ppm),  $C_{\text{chamber}}$  is the chamber CO<sub>2</sub> mole fraction, and  $\tau$  is the leak rate time constant (s). A simple leak test can be performed by first reducing the chamber CO<sub>2</sub> mole fraction to 50–100 ppm using the system CO<sub>2</sub> scrubber, and then measuring the rate of CO<sub>2</sub> increase ( $dC_{\text{chamber}}/dt$ ) with a filter paper leaf replica in the chamber. Since the chamber CO<sub>2</sub> mole fraction is always known, and the ambient CO<sub>2</sub> mole fraction is constant and easily measured,  $\tau$  can be computed. We have found that  $\tau$  is constant and independent of the CO<sub>2</sub> gradient for a given set of conditions. Once  $\tau$ ,  $C_{\text{chamber}}$  and  $C_{\text{ambient}}$  are known, the leak rate can be computed and subtracted from the measured CO<sub>2</sub> change rate. The LI-6200 can be programmed to calculate the leak rate

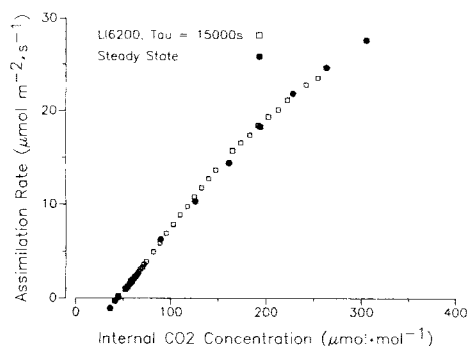
and correct each assimilation measurement as the chamber CO<sub>2</sub> mole fraction declines. Both corrected and uncorrected data can be stored.

As the experiments reported in Figs. 2–5 progressed,  $\tau$  declined from about 15 000 s to about 7000 s, presumably due to chamber gasket deterioration. The effects of leaks on the LI-6200 data from Fig. 2 are shown in Fig. 3 for 2 values of  $\tau$ . Chamber leaks have important effects at low chamber CO<sub>2</sub> mole fractions, but negligible effects at ambient levels. In ordinary photosynthesis measurements where CO<sub>2</sub> concentrations are near ambient, only small gradients exist to drive CO<sub>2</sub> diffusion into the chamber, so chamber leaks are not a problem. However, when CO<sub>2</sub> response curves are being measured, leak tests should be performed regularly, and the data corrected accordingly. Fig. 4 shows the LI-6200 data from Fig. 2 after the leak correction was applied. The correspondence between the steady state and LI-6200 results is excellent. Similar results were obtained in a 2nd experiment.

CO<sub>2</sub> response curves for 2 separate leaves of chamber-grown cotton were measured late in the afternoon. Leaves were trimmed symmetrically about the



**Fig. 3.** LI-6200 data of Fig. 2 recalculated to account for a chamber leak with a time constant of 7000 or 15 000 s.

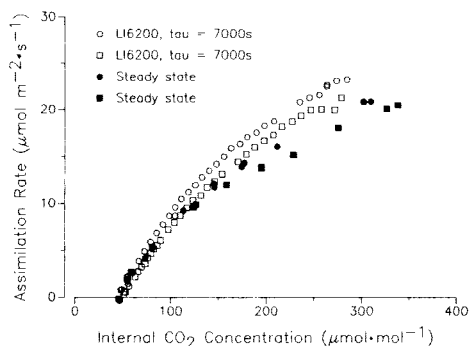


**Fig. 4.** LI-6200 data of Fig. 2 after leak correction with a 15 000 s time constant compared to the same steady state data shown in Fig. 3.

mid-vein prior to measurement. LI-6200 data were first obtained in the growth room, and then the plants were transferred into fresh growth solution, taken down a cool, dimly lit outside hallway and into the laboratory, where steady state measurements were performed. Results for both the steady state system and LI-6200 are shown in Fig. 5. Compensation points and initial slopes are in excellent agreement, but maximum rates were higher when measured *in situ* with the LI-6200. There is little doubt that the time of day and prior treatment of the plants affected maximal rates measured with the steady state system.

## Discussion

These and other experiments support the conclusion that well-watered C-3 plant leaves are able to maintain a quasi-steady state with respect to CO<sub>2</sub> mole fractions which change at the rates observed in typical experiments (*e.g.*, 0.01–1 ppm·s<sup>-1</sup>). Under these conditions, the transient approach provides a valid method for measuring CO<sub>2</sub> response



**Fig. 5.** Comparison of response curves measured *in situ* on chamber-grown cotton using the LI-6200 (open symbols) with measurements on the same leaves in the laboratory using the steady state system (closed symbols). Conductances varied in the range 0.43–0.81 and 0.44–0.99 mol·m<sup>-2</sup>·s<sup>-1</sup>, (LI-6200), and 0.23–0.43 and 0.19–0.41 (steady state system). Average leaf temperatures were 31.9°C (LI-6200) and 31.7°C (steady state).

curves. It is rapid and convenient inasmuch as it does not require a series of mixed gasses or long equilibration times, and it can be performed with a compact and portable instrument. However, a major question which remains is leaf temperature control.

Leaf temperature control in the LI-6200 chamber relies on evaporative cooling of the leaf and passive heat exchange with the environment. Since there is no active temperature control, leaf temperature increases, which might occur during a measurement lasting 20 min or more, are a matter of concern. As indicated in the figure legends, leaf temperature control in artificial environments is not a serious problem. High intensity incandescent lamps which produce a narrow light beam can be filtered with a dichroic mirror. Such a light source was used to produce the data of Figs. 3–5. Clear plexiglas makes an excellent IR filter for high intensity discharge lamps. A plexiglas filter, along with an external fan and water-cooled

luminaire, effectively controlled leaf temperature increases under our HID lamp.

The problem is more serious in the field, although it is not insurmountable. Davis *et al.* (1987) reported a chamber temperature increase of only 1.3°C while measuring a CO<sub>2</sub> response curve on green ash under full sun (1750 μmol·m<sup>-2</sup>·s<sup>-1</sup>, 35°C). In many cases, moderate chamber and leaf temperature increases of 2–3°C occur during a measurement in full sun. Under unfavorable conditions, temperature increases of up to 6°C have been observed; this, of course, is unacceptable. Keeping the chamber cool and shaded when not in use, and adequate transpiration rates, help to moderate temperature increases.

The infrared filters that work so well under artificial lights do not help very much in the field because plant leaves have relatively little absorptance in the near IR, and the solar spectrum has relatively little energy in the longer wave regions. However, an external fan does a surprisingly good job of moderating chamber temperature increases. One of us (JMN) found that when a Big Blue Stem (*Andropogon gerardii* Vitman) leaf of about 5 cm<sup>2</sup> was enclosed in the 1/4 l chamber at an outside air temperature of 40°C, the chamber air temperature remained near

41°C with an external fan, whereas the chamber air temperature gradually increased to 44°C without the fan. With proper techniques, temperature increases can often be held to under 2–3°C. The data of Brooks and Farquhar (1985) on spinach indicate that a 2°C temperature increase at 30°C would cause a 7% increase in the photorespiratory CO<sub>2</sub> compensation point.

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