Measurement of Photosynthetic Gas Exchange in Controlled Environments

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Early controlled-environment research used growth chambers primarily as "photoperiod rooms" to study effects of environment on long-term plant developmental processes (Downs and Hellmers, 1975). More recently, growth dynamics analysis has been used to measure photosynthetic productivity of crop canopies or individual plants in controlled environments over periods of only a few days (Knight and Mitchell, 1983). However, as the emphasis of controlled-environment research shifts toward definition of optimum growth conditions (Hoff et al., 1982), there will be increasing demand to measure instantaneous photosynthetic response within modified controlled environments.

Net photosynthesis and respiration are measured most conveniently as CO_2 consumption or evolution, respectively, by a given area or mass of plant tissue over a defined period. At $\approx 350 \mu\text{mol}\cdot\text{mol}^{-1}$ ($\mu\text{l}\cdot\text{liter}^{-1}$, vpm), CO_2 is a trace gas compared to the major components of air (N_2 , O_2 , H_2O). As such, CO_2 is detectable to $1 \mu\text{mol}\cdot\text{mol}^{-1}$ by thermal conductivity gas chromatography (TCD-GC) or infrared gas analysis. The infrared gas analyzer (IRGA) is amenable to continuous-flow monitoring of the CO_2 level in air and will detect near-real-time changes in gas-exchange rate by plants as environmental conditions vary. Modern electronic detector systems permit a degree of miniaturization of IRGAs that allows their portability into growth chambers as well as to the field.

Gas exchange by chamber-grown plants may be measured for individual attached leaves, entire shoots, or leaf canopies of multiple plants. Various plant enclosure techniques are used for each type of measurement. Because a limited number of off-the-shelf assimilation chambers are available on the commercial market, many researchers have developed their own cuvettes for specific applications. Assimilation chambers often are constructed from transparent acrylic (plexiglass) or polycarbonate (mylar) plastic. Because these plastics adsorb water vapor (H_2O) and, consequently, CO_2

(Bloom et al., 1980), it is advisable to coat internal chamber surfaces with transparent teflon tape. Closed-cell foam frequently is used to seal petioles, individual leaf blades, or fixed areas of leaves within a cuvette. It is important that gasket material be soft and flexible since physical pressure on lamina, petioles, and stems during sealing may significantly impair translocation, cause stomatal closure, and inhibit growth (Geiger, 1974; Mitchell et al., 1975; Pappas and Mitchell, 1985). Most assimilation chambers rely on ambient growth chamber lighting to drive photosynthesis.

Closed systems

The simplest assimilation chambers are hand-held, clamp-on devices adapted for withdrawal of air samples to be subsequently injected into a GC or IRGA. If lacking in temperature control, they rely on rapid CO_2 depletion by the enclosed leaf to provide a detectable change before significant "greenhouse effect" heating occurs. Most procedures traditionally permit $< 10\%$ drawdown of CO_2 from ambient chamber air enclosed with a leaf or plant before the depletion is considered to be significant (Lang and Hällgren, 1985). Such measurements typically take from several seconds to less than a minute, depending on the size of the assimilation chamber relative to the exposed area of enclosed tissue. Manual syringe pumping can be used to mix enclosed air immediately before sampling, or the cuvette may contain a battery-powered fan that is activated by closing the lid. Parallel arrays of fishing line can be used to support the leaf in an appropriate orientation within the cuvette without interfering with air circulation and will prevent contact with fan blades during leaf flutter. If air samples are injected into a TCD-GC, H_2O of transpiration can be separated chromatographically from CO_2 before the latter enters the detector, but if analyzed by IRGA, this cannot be done. Since H_2O absorbs in some of the same infrared wave bands as CO_2 (e.g., at $2.7 \mu\text{m}$), air may be passed through a non- CO_2 -absorbing desiccant such as $\text{Mg}(\text{ClO}_4)_2$ or ZnCl_2 before entering the sample cell of the IRGA. Otherwise, CO_2 concentration in the air sample could be significantly overestimated. Newer IRGAs are equipped with optical filters to separate H_2O absorbance from that of CO_2 , thereby eliminating the need to

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redry air. For closed systems in which contained air is circulated between an assimilation chamber and an IRGA, a partial gas-flow bypass through a desiccant may be required to prevent accumulation of transpiration H_2O in the assimilation chamber (Fig. 1A). Several commercial photosynthesis systems are portable, closed systems having a built-in, miniature IRGA and automatic humidity compensator. To accommodate portability, they usually do not have internal temperature control, but rely on rapid depletion of CO_2 in the leaf cuvette for gas-exchange measurement. As for all closed systems, all seals must be air tight, the volume of the cuvette and associated plumbing must be known, and measurement must be rapid enough to minimize effects of changing temperature and/or CO_2 concentration on the gas-exchange rate. Assimilation rate in a closed system is given by $P_n = (C_i - C_o)/(t_2 - t_1) \times v \times 1/L$ (Sestak et al., 1971), where P_n = photosynthetic assimilation rate (amount of CO_2 consumed per unit leaf area per unit time; e.g., $\mu mol \cdot m^{-2} \cdot s^{-1}$); C_i and C_o = CO_2 concentration at times t_1 and t_2 (e.g., $\mu mol CO_2/mol$ atmosphere); v = total system volume (m^3); and L = leaf area (m^2). The measured photosynthetic rate should be corrected to standard temperature and pressure (STP is 0°C or 273K and 1 atm or 0.1013 MPa). For example, a liter of gas at 25°C has 9.2% fewer moles than a liter of the same gas at standard temperature (0°C). Without correction for temperature and pressure (especially altitude), gas-exchange rates for similar plant material would vary widely for different parts of the world.

Semiclosed systems

Another mode of measuring gas-exchange rates in growth chambers involves use of semiclosed systems. In this mode, gas recirculates between the plant enclosure chamber and IRGA, but instead of allowing CO_2 to deplete, as with a completely closed system, CO_2 is injected into the system at a rate that balances the rate of CO_2 consumption by the plant (Fig. 1B). This "null balance" is maintained with a mass-flow controller that receives signals from the IRGA and in turn signals a proportioning valve to modulate the flow of CO_2 from a compressed gas cylinder into the system. Alternatively, a solenoid valve can be actuated to keep the CO_2 level within tight limits. The obvious advantage of a semiclosed system is that P_n can be measured at a steady-state level of CO_2 in the assimilation chamber. Thus, $P_n = f/L$ (Long and Hällgren, 1985), where f = flow rate of CO_2 into the system (e.g., $\mu mol \cdot s^{-1}$) and L = leaf area (m^2) or mass (g). As for a completely closed system, transpiration H_2O should be partially absorbed with a bypass drier. The IRGA used in a semiclosed system must be an absolute rather than a differential instrument since there is no reference airstream to bypass the assimilation chamber, as with an open system. The additional complexity of a semiclosed system precludes the portability of simpler closed systems. The leaf cuvette or plant enclosure chamber is connected to the IRGA, to pumps that move air, and to flow controllers, driers, and CO_2 cylinders. To avoid cluttering the growth chamber and exposing electronic instruments to high humidity in the growth area, most electronic equipment can be located outside the growth chamber, connected to the assimilation chamber by wires and tubing passing through ports.

A potential drawback of using semiclosed systems is that the investigator might be tempted to enclose plants in chambers for extended P_n measurement, since CO_2 depletion would not occur. Prolonged enclosure without adequate provision for control of rising O_2 concentration or scrubbing of volatile outgassing components, such as H_2O and ethylene, could have a negative impact on validity of P_n measurements.

Open systems

Another type of gas-exchange system used in controlled environments is an "open" system, in which atmosphere flows through an assimilation chamber, usually after being thoroughly mixed by an internal fan before exiting the chamber at a fixed rate (Fig. 1C). A portion of the outlet gas is directed through an IRGA before being exhausted from the system, preferably out of the controlled environment entirely. The flowing atmosphere may come from one or more compressed gas cylinders, or from a pump or air compressor

Fig. 1. Schematic representation of typical systems for measuring photosynthetic gas exchange, including (A) closed, (B) semiclosed, (C) open without CO_2 injection, and (D) semi-open with CO_2 injection. Lines with arrows depict direction of atmosphere flow. Heavier lines in (C) denote more rapid rates of flow than in (A) or (B), and dotted arrows represent ambient air being taken up by a pump in open systems. (c) Cuvette or assimilation chamber, (d) bypass drying column used to prevent build-up of humidity in closed systems. CD infrared CO_2 gas analyzer, either a differential instrument (sample + reference inlet/outlet) or an absolute measuring device (sample inlet/outlet only + sealed reference bench), (g) CO_2 compressed gas cylinder, (m) mass-flow controller, (p) metal bellows pump (especially for closed systems), (v) mass-flow control valve.

delivering ambient atmosphere. Depending on the irradiated area of photosynthetic tissue within the assimilation chamber, turnover rate of atmosphere may have to be fairly rapid to prevent unwanted drawdown of CO_2 using an open system without CO_2 injection.

The IRGA usually is operated in a differential mode for an open system, although measurement in an absolute mode is feasible with appropriate bypass lines and valves. In a differential mode, the airstream is split before entering the cuvette, and one line bypasses the plant chamber altogether. The outlet airstream passes through the sample cell of a differential IRGA, while the bypass airstream passes through the reference cell. The IRGA detects the net CO_2 concentration difference between sample and reference cells. If an absolute IRGA is used, sample and bypass airstreams are read alternately, with the one not read being vented from the system. Gas used in open systems may need to be humidified before entering the plant cuvette, because turnover rate must be rapid enough that CO_2 depletion is not large, and outlet gas may or may not have to be redried before entering the IRGA, depending on the sensitivity of the IRGA to H_2O . The assimilation chamber of an open system often is air-conditioned to help maintain steady-state gas-exchange rates. A difference in CO_2 concentration between inlet and outlet airstreams of only a few micromoles per mole is needed to determine steady-state gas-exchange rate, which is expressed as $P_n = f(C_i - C_o)/L$ (Sestak et al., 1971), where C_i = inlet CO_2 concentration, C_o = outlet CO_2 concentration, f = flow rate of air through the system, and L = leaf area or mass.

Semi-open systems

A specialized type of open gas-exchange system involves slow turnover of the atmosphere but separate injection of CO_2 to maintain a steady-state CO_2 level (Fig. 1D). This type of open system uses far less ventilating gas than does an open system. Minitron chambers (Akers et al., 1985; Knight et al., 1988) may be adapted to operate in various modes, including semi-open. Single or several plants can be grown hydroponically in Minitrons and shoot and root compartments sufficiently isolated from each other to measure gas exchange in both compartments separately (Fig. 2). An internal air circulation/cooling system, including fan, heat-exchange coils, and baffle, prevents stratification of air and temperature within cham-

Fig. 2. Schematic side view of a Minitron II chamber. The cylinder is 63.5 cm high with a 61.2 cm o.d. and has an internal volume of 0.151 m³, excluding internal accessories. (A) lid, (B) base, (C) cylinder, (D) height-adjustable, threaded metal legs, (E) tension springs, (F) air-flow baffle, (G) height/position-adjustable thermistor, (H) hydroponics container, (I) hydroponics container platform, (J) plant holder with closed-cell plug in middle, (K) automobile core heat exchanger, (L) heat-exchanger platform, (M) outward-sloping floor, (N) fan motor, (O) fan blades, (P) bulk-head fittings for portals in base, (Q) condensate drain from chamber, (R) rhizosphere outlet, (S) nutrient solution inlet, (T) shoot atmosphere outlet, (U) inlet for nutrient solution aeration, (V) coolant inlet, (W) coolant outlet, (X) nutrient solution outlet, (Y) shoot atmosphere inlet (Knight et al., 1988).

bers. To achieve CO₂ enrichment, separate mass-flow control systems inject CO₂ and air into a mixing chamber upstream from the inlet to the shoot compartment (Fig. 3), although CO₂ injection theoretically could take place directly into an assimilation chamber in which air is well stirred. Outlet atmosphere can be monitored for water vapor content by dewpoint hygrometry and for CO₂ by infrared gas analysis. A computer-assisted, mass-flow control system coupled with an IRGA can maintain steady-state CO₂ concentration within Minitron chambers using a proportional-integral-derivative control program. Carbon dioxide consumption rate is proportional to the voltage signal required to drive a proportioning valve to an aperture that maintains steady-state CO₂ level in the chamber containing photosynthesizing plants. Canopy gas exchange is determined as the difference in voltage signal to the proportioning valve required to maintain a given CO₂ concentration in an empty Minitron with a slow turnover atmosphere and one containing plants. An advantage of such a system is that plants can be measured for gas exchange in the very environment in which they grow, without changing environmental conditions and without clamping a cuvette around the leaves. The assimilation chamber is the growth chamber.

Lighting considerations for gas exchange in growth chambers

Lighting for transparent gas-exchange cuvettes or assimilation chambers can be provided by lamps mounted on the ceiling of a controlled-environment room housing the system, or by lamp banks mounted above the enclosure chamber(s). An advantage of using "ambient" growth chamber lighting for gas-exchange measurement

is that it is representative of the radiation environment to which the plants already are adapted. However, construction material for enclosure chambers (plastic, glass, etc.) will modify the spectral quality of incident radiation from lamps in growth rooms. Both long-wave and ultraviolet radiation can be attenuated by chamber wall and lid material. Special UV-transmitting acrylics can be obtained for constructing enclosure chambers. If high irradiance is used to drive photosynthesis, lamp radiation can be filtered through long-wave-absorbing material or several centimeters of water to remove thermal wave bands that otherwise would enter assimilation chambers. This thermal filter precludes a much greater heat load on any internal air-conditioning system, thereby allowing heat-exchange coils to be maintained at a temperature closer to ambient and causing less dehumidification resulting from condensation of water in the chamber.

General considerations for gas-exchange measurement

If one wishes to monitor photosynthesis under the full spectrum of lamp radiation unattenuated by assimilation chamber surfaces, it is possible to adapt the growth chamber itself to be a large gas-exchange cuvette. By rigorously sealing all potential leak points in the chamber (e.g., with silicon sealant), installing an inlet port for make-up gas and an outlet port and pump to an IRGA, and maintaining the chamber under slight positive pressure, it is possible to monitor net photosynthesis of the-contained crop canopy (Bugbee and Salisbury, 1986). A good internal air-circulation system is important to assure that sampled atmosphere is representative. Since the presence of a single person in a walk-in growth room can raise the CO₂ level hundreds of parts per million in several minutes, access by personnel to the interior of a walk-in or stand-in chamber adapted for gas exchange normally must be denied during measurement if the system is to be operated near normal ambient. However, if human presence in such an adapted chamber cannot be avoided, exhalation breath must be absorbed by a face mask and/or directed out of the chamber through a hose. Furthermore, skin respiration would require full suit enclosure of workers to avoid, perturbation of CO₂ level in the chamber atmosphere for precise work.

Fig. 3. Schematic of Minitron II environmental control and monitoring systems. (A) chamber, (B) root compartment, (C) thermostat-controlled, circulating water bath (Lauda model K-4R), (D) air compressor (Gast model SHCD), (E) air-drying cylinder containing CaCl₂ pellets, (F) air-purifying tower containing Purafil to absorb ethylene, (G) mass-flow controller (MKS model 1259A), (H) humidification cylinder, (I) CO₂ cylinder (99.95% CO₂), (J) microcomputer (Action Instr. model BG-3), (K) calibration gas standards, (L) infrared gas analyzer (Horiba model PIR-2000), (M) rotameter (Matheson model 603), (N) needle valve, (O) dewpoint hygrometer (EG&G model 911), (P) dewpoint remote sensor, (Q) scanning telethermometer (YSI model 47), (R) three-way valve, (S) air-drying cylinder containing Mg(ClO₄)₂, (T) booster pump (Metal Bellows model MB-21) (Knight et al., 1988).

Ability to monitor net photosynthesis of crop canopies in growth chambers under different combinations of environmental conditions will lead to the rapid establishment of response surfaces for optimum productivity during vegetative and reproductive phases of development for some crops, based on instantaneous gas-exchange measurements rather than long-term growth or yield.

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