Chapter 4

Carbon Dioxide

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Contents

Introduction

Light and water for plant growth are provided for in all growth chamber designs because the need for these raw materials of photosynthesis is well known. Less obvious, however, are the need for carbon dioxide (CO_2) and the effects of various CO_2 concentrations on plant growth. Klueter (1979) described CO_2 as one of the least controlled factors in plant growth chambers. Whereas unlit plants turn yellow and unwatered plants wilt, CO_2-starved plants merely cease to grow, or grow more slowly. Growth responses to elevated CO_2 and symptoms of excessive CO_2 on plants are also hard to detect except in comparison with paired plants growing at ambient CO_2 levels. Perhaps CO_2 control receives less attention in growth chamber design because human senses are well adapted to perceiving light and water but are incapable of detecting CO_2, even at lethal levels (Kling et al., 1987). For whatever reason, researchers are often unaware of the problem of CO_2 effects in growth chambers (Bernier et al., 1994). Growth chambers and rooms constructed before 1984 usually offer little in the way of monitoring or controlling CO_2 concentration. Air exchange with the outside is often limited (Bernier et al., 1994).

Only recently have commercial growth chambers been available with built-in CO_2 controls. This chapter is written to assist users of growth chambers without built-in CO_2 controllers in implementing at least a minimum level of CO_2 control. We also hope to help users of systems with built-in CO_2 controllers to understand and manage their systems. Although many types of chambers, including open-top units (e.g., Drake et al., 1985), are used in studying CO_2 effects on

plants in the field, the discussion in this chapter is confined to CO_2 control in commercially available, closed chambers.

Problems in Regulation

People-related

Although the CO_2 concentration of empty chambers may vary seasonally or yearly, by far the most serious problem in CO_2 control results from human activity. A person in a chamber exhales air that is 4-5% CO_2. Within a few minutes, the amount of CO_2 in the chamber may increase more than tenfold, far above the normal atmospheric level of 350 μmol mol^{-1}. These elevated levels may persist for some time, particularly if the rate of fresh air exchange is low or if relatively few actively photosynthesizing plants are present in the chamber. As summarized by Bernier et al. (1994), the effect of these increased CO_2 concentrations on the plants is greatest with plant measurement of such CO_2-dependent physiological processes as transpiration, stomatal conductance, water use efficiency, xylem water potential, photosynthesis and dark respiration. Growth and carbohydrate levels should be less affected by short-term CO_2 increases.

Solutions to human CO_2 pollution in chambers are generally cumbersome. Stewart and Bernier (1994) observed that when an investigator entered one of their growth chambers, the CO_2 concentration rose by 300 μmol mol^{-1} within 20 minutes even though the chamber had an air exchange rate of about 3.5 times per hour. To reduce potential effects on the plants, they developed a mask/vacuum system to exhaust the air. Molded plastic respirator masks were purchased from an industrial safety supplies store. They then reversed the removable inlet and outlet valves so that air could be freely inhaled (through what was normally the outlet), and air exhaled out through the cartridge outlet. One end of the cartridge was cut away, but the cartridge was left in place because standard plumbing fixtures could not be attached directly to the proprietary connectors on the masks. They glued a plastic elbow joint onto the cartridge and attached corrugated plastic hose to the elbow joint with a hose clamp. Plastic laboratory tubing was then used to connect the chamber with the building vacuum line. The ends of both the corrugated plastic hose leading from the mask and the plastic laboratory tubing leading from the building vacuum system were brought into a large plastic bag and the ends sealed with adhesive tape. The bag provided an expandable buffer volume for the system and reduced the amount of suction necessary to exhaust the CO_2.

The authors report that this system essentially eliminated human CO_2 contamination of the chamber, while providing reasonable comfort for the wearer. The suction available from their system, approximately 50 liters/min, was sufficient to draw air away from one mask rapidly enough to keep the wearer's face cool and dry. When two masks were connected to the system, however, the masks were less comfortable. The authors also caution that chamber exhaust must be discharged outside the building, or at least some distance from the chamber, so that it will not leak back in. For facilities lacking a central vacuum, they suggest using an air pump, such as a vacuum cleaner.

Free-standing commercial chambers have leaks in joints and connections that allow air exchange in addition to the "fresh air" intake. Leak rates can be quite variable among chambers and even among chambers of the same type; each should be checked individually for leak rate (Bernier et al., 1994). In one reach-in chamber at the University of Wisconsin, the leakage was equal to 30 % of the internal volume in 5 minutes even though the fresh air intake fans were sealed off (Tibbitts and Krizek, 1978). Thus if leakage is high, the CO_2 level in "controlled"

environment chambers will closely track human activity or other sources of CO_2 in surrounding areas. This is particularly undesirable when chambers are located next to potting or coffee break areas with their periodic fluctuations of activity. Acock and Acock (1989) and Bernier et al. (1994) describe methods for calculating air leakage rates in controlled environment chambers. Once this rate is calculated, equations provided by Bernier et al. (1994) can be used to determine the approximate effect that observers have on their own experiments. As Bernier et al. point out, however, these equations assume constant ambient CO_2 concentrations, which is rarely the case in modern energy-efficient buildings.

Plant-related

Plants cause two types of problems in maintaining CO_2 concentrations. During the night, plant respiration can more than double the CO_2 concentration in an unventilated chamber. Elevated CO_2 concentrations during the dark have been shown to stimulate biomass production in the early phase of growth of soybean plants (Bunce, 1995). Since total leaf area and leaf photosynthetic rates were not increased by elevated nighttime CO_2, the increase in biomass and net assimilation rate was attributed to reduced CO_2 efflux during the dark. The daytime drawdown of CO_2 by plant photosynthesis is better documented than the nighttime buildup. Daytime CO_2 drawdown poses a serious limitation to plant growth if CO_2 levels fall far enough below ambient levels to reduce growth. A typical example is shown in Fig. 1 for a crop of wheat plants growing in NASA's Biomass Production Chamber at the John F. Kennedy Space Center (Wheeler, 1992). At 25 days after planting, the CO_2 level was raised to 2300 µmol mol^{-1} and then allowed to draw down to near the compensation point. As shown in Fig. 1, the rate of the CO_2 drawdown was relatively constant from 2300 µmol mol^{-1} down to approximately 800 µmol mol^{-1}. Below this level, the rate of the CO_2 drawdown decreased gradually, indicating an increasingly slower rate of photosynthesis at the lower CO_2 concentrations. As another example, Bernier et al. (1994) reported that *Populus* grown inside a walk-in growth chamber lowered the CO_2 concentration from the level of 400 µmol mol^{-1} found inside the research facility to 280 µmol mol^{-1}, a drawdown of 120.

Problems of excessive or deficient levels of CO_2 are most acute in built-in rooms that have very little leakage and may be totally dependent upon "fresh air" intake fans. For some crops and chambers, the amount of outside air that can realistically be brought in may be insufficient to maintain acceptable CO_2 concentrations without the injection of additional CO_2. Patterson and Hite (1975) reported that introduction of outside air (1% of chamber volume per minute) failed to maintain ambient CO_2 concentration in the plant chambers at the Duke University Phytotron. Cotton plants lowered the CO_2 concentration to 150 µmol mol^{-1} and corn to 50 µmol mol^{-1} even though outside air contained 350 µmol

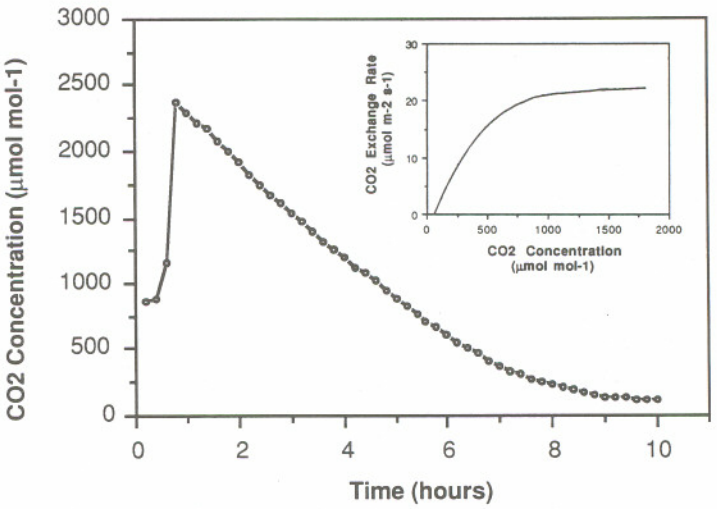

Figure 1. Photosynthetic drawdown of carbon dioxide by a stand of wheat growing in NASA's Biomass Production Chamber at the John F. Kennedy Space Center. At 25 days after planting, the CO_2 concentration was raised to 2300 µmol mol^{-1} and then allowed to draw down to near the compensation point (Wheeler, 1992).

$mol^{-1} CO_2$. Calculations made by Tibbitts (Tibbitts & Krizek, 1978) showed that air exchange rates must equal one chamber volume each 1-2 minutes to avoid significant CO_2 depletion when a chamber is filled with photosynthesizing plant tissue. If CO_2 concentrations are depleted over a long period of time, reductions in dry mass production and growth rates are to be expected.

Changes in Ambient CO_2

Ambient concentrations in atmospheric CO_2 have changed markedly during the past 50 years (Allen, 1990; Conway et al., 1988; Idso, 1989; Krizek, 1989; Krupa and Kickert, 1989), making it difficult to decide on where to set the "control" or "ambient" CO_2 concentration. Since 1940, the ambient CO_2 concentration has increased from approximately 300 μmol mol^{-1} to approximately 350 μmol mol^{-1}. Within the next 80 years, the CO_2 concentration is predicted to reach 600 μmol mol^{-1} (Gammon et al., 1985). Such changes in ambient CO_2 level from year to year make it difficult to maintain a constant or "benchmark" CO_2 concentration in the growth chamber unless a CO_2 scrubbing system is employed or progressively higher baseline levels of CO_2 are used each year.

Seasonal variations in atmospheric CO_2 levels may also occur. In general, CO_2 concentrations are higher in winter than in summer. In urban areas, ambient CO_2 concentrations often are elevated 50 μmol mol^{-1} or more as a result of inversions that trap air over the city. When growth chambers are located inside buildings, less outside air is brought in to cool the chambers during the winter heating or summer cooling seasons, and workers and machinery will raise levels inside the building. Without sufficient outside air exchanges, growth chamber CO_2 concentrations tend to track the CO_2 levels immediately around the chamber, which often reach 400 μmol mol^{-1} or higher. This can also result in seasonal variation in chamber CO_2 concentrations. Seasonal CO_2 variations pose a particular problem in growth chambers because an important rationale for using growth chambers is freedom from seasonal effects. This freedom, real or imagined, has encouraged investigators to repeat their experiments over time if, as is often the case, insufficient chambers were available to replicate all treatments at once.

Table 1. Response of crop plants to an increase in CO_2 concentration above current ambient level (summarized from Acock and Allen, 1985).

Process	Effects on Plants
Leaf photosynthetic rates	Increases in all plants on first exposure. C3 respond more than C4. Little response above 1000, and levels above 2000 may be toxic.
Inhibition of photosynthesis by source-sink imbalance	Response occurs in many species.
Leaf transpiration rate	Decrease in all plants. C4 plants respond more than C3.
Leaf anatomical and biochemical adaption	Leaf area, weight per unit area, thickness, and number of mesophyll cell layers increase in many species.
Canopy leaf area	Usually increases.
Carbon partitioning among organs	Proportion of carbon going to roots and stems is increased in many, but not all, species.
Branching, flowering and fruiting	Initiation and/or retention of these organs is increased in many species
Fruit and seed	Increases in number and/or size of fruits and seeds.
Canopy water-use efficiency	Increases in C3 and C4 plants. Increase in photosynthesis or yield contributes more than reduction in transpiration.
Yield	Increases 32% on average between 300 and 660 μmol mol^{-1} for plants in favorable conditions.

Plant Responses

Recent research and reviews on plant response to elevated CO_2 (e.g., Acock and Allen, 1985; Allen, 1990; Cure and Acock, 1986; Enoch and Kimball, 1986; Idso, 1989; Kimball, 1983, 1986a, 1986b; Krizek, 1986, 1989; Krupa and Kickert, 1989; Lemon, 1983; Strain and Cure, 1985) have shown that plant response to elevated CO_2 varies with species, developmental stage, irradiance, temperature, mineral nutrition, and

possibly size of the rooting container (Thomas and Strain, 1991). What was once thought to be a straightforward increase in photosynthesis, and therefore growth, with increasing CO_2 is now known to involve a complex series of physiological, metabolic, and morphological changes. These effects are summarized in Table 1.

Whatever the complexities of plant response to higher-than-ambient levels of CO_2, of great concern to growth chamber users is the well-established fact that CO_2 concentrations below ambient levels decrease photosynthesis and plant growth. Physiological changes undoubtedly also occur at low CO_2 concentrations, but these changes are not well documented. Few data are available on the effects of lower-than-ambient CO_2 concentration on plant growth (Allen et al., 1991) because of the difficulties of scrubbing CO_2 from chamber air to maintain these concentrations. A greenhouse study by Heij and van Uffelen (1984) illustrates the sensitivity of crop growth to CO_2 concentrations below ambient. When below-ambient CO_2 concentration was raised 50 µmol mol^{-1} (from 100 µmol mol^{-1} to 150 µmol mol^{-1}), cucumber production increased 26.4%, but when concentrations were raised 50 µmol mol^{-1} above ambient (from 350 µmol mol^{-1} to 400 µmol mol^{-1}), the yield increase was only 3.6%. Allen et al. (1991) report a similar sensitivity of dry matter production in soybean to below-ambient CO_2 concentrations.

Nature

Carbon dioxide (or carbonic anhydride) is a nonflammable, colorless, odorless gas at room temperature, and a volatile, colorless liquid or a white snow-like solid subliming below -78.5 °C. It has a molecular weight of 44.01 and is approximately one and one-half times as heavy as air. Because CO_2 is heavier than air, if the CO_2 concentration is high enough in an air-CO_2 mixture, it will settle out. This is not a problem with the normal range of CO_2 concentrations used, however. Tanks containing mixtures of CO_2 and other gasses are rolled by the manufacturer before analysis and will remain stable for at least 5 years without further mixing. Only when CO_2 concentrations approach 50% does settling out become a problem. Carbon dioxide comprises approximately 0.034% of dry air by volume and approximately 0.052 % by weight, so a concentration of 50% is far higher than the normal range used in growth chambers. CO_2 at this concentration would, in fact, be toxic. At sea level pressure and 15 °C, one volume of CO_2 gas will dissolve in approximately one volume of water. Its solubility in pure water at 0 °C is twice that at 20 °C and nearly three times that at 30 °C (Šesták et al., 1971). nder conditions normally encountered in the laboratory, CO_2 is a very stable compound.

Sources

Liquid in Pressurized Cylinders

When carbon dioxide is added to controlled environments, it is usually provided as a liquid in pressurized metal cylinders; cylinders weigh from 20 to 75 pounds and are shipped back to the supplier to be refilled. Four grades of CO_2 can be provided in the refillable cylinders: research (99.995% minimum purity); instrument (99.99% minimum purity); bone dry (99.8% minimum purity); and commercial (99.5% minimum purity). Typical specifications for research grade would be N_2, 6 µmol mol^{-1}; O_2, 6 µmol mol^{-1}; water, < 20 µmol mol^{-1}. For instrument grade, typical specifications would be N_2, 50 µmol mol-^{1}; O_2, 4 µmol mol^{-1}; water, 10 µmol mol^{-1}; dew point, -76 °F. For the bone dry grade, specifications would be: N_2 and O_2, 0.05%; dew point, -30 °F; and oil content less than 5 µmol mol^{-1}. Typical specifications for commercial grade would be N_2, 0.34%; O_2, 0.09%; and water, 0.07%.

To raise the CO_2 concentration in a plant

growth chamber, we recommend using commercial grade because it is the most reasonably priced and is quite adequate. For calibration of infrared analyzers, however, instrument or bone dry grade is recommended. Very large users of CO_2 may find it more economical to buy commercial grade CO_2 in large storage tanks, refilled on the site, but this involves considerable expense in constructing a pad and buying or leasing the container. The purity of such bulk supplies also is more difficult to guarantee but may be adequate for growing areas with extensive ventilation such as large greenhouse ranges or open-top chambers.

Problems with contaminants in the CO_2 and from the cylinders have sometimes been reported. In most cases, the contaminants are a by-product of the processes used to generate CO_2. When CO_2 is obtained from underground wells, it is usually clean, but when acetylene is used as the CO_2 source, ethylene is an occasional contaminant. The following example of "typical" tank gas composition was provided by L. Giles at the Duke University Phytotron for CO_2 produced as a by-product of anhydrous ammonia production from methane: CO_2, 99.95%; H_2O, < 16.2 μmol mol^{-1}; H_2S, < 1 μmol mol^{-1}; NO, < 5 μmol mol^{-1}; SO_2, < 5 μmol mol^{-1}; CO, < 10 μmol mol^{-1}; and NH_3, < 25 μmol mol^{-1}.

The cylinder itself is also a potential source of contamination. The frequency of replenishment of the CO_2 cylinder and the type of metal used in the cylinder are important factors in determining the extent of contamination. Precision calibration mixtures should be supplied in aluminum cylinders to minimize corrosion over time. Steel cylinders can be used for CO_2 addition or minimum accuracy calibration mixtures. If the steel cylinders have not been used for some time, it is a good idea to send them back to the manufacturer for cleaning and inspection for corrosion.

Flow of CO_2 from the cylinder into the chamber must be precisely controlled with automatic pressure regulators. There are three main types: One- or two-stage automatic pressure regulators and low-pressure regulators. The single-stage regulator reduces cylinder pressure in one step to a range of delivery pressures. Delivery pressures are available in ranges from 0.22-0.55 to 0.69-10.34 MPa (4-80 to 100-1500 psi). As cylinder pressure falls, a single-stage regulator will show a decrease in delivery pressure, but since CO_2 is a liquefied gas at room temperature, the cylinder pressure will remain reasonably constant as long as any liquid CO_2 remains in the cylinder. Thus, a steady delivery pressure will be produced until approximately 80 percent of the CO_2 in the cylinder has been discharged. Temperature is also important. Above the critical temperature of 31 °C, CO_2 converts completely to a gas, so the discharge of gas will show a steady drop in pressure. The two-stage regulator performs the same function as a single-stage regulator, but the second regulator allows the change in delivery pressure and flow to be monitored as the cylinder pressure decreases. In automated systems, to control CO_2 release from gas cylinders, a two-stage regulator is sufficient. For a constant delivery pressure, a low-pressure regulator must be used in combination with a two-stage regulator. The use of gauged two-staged and low-pressure regulators also provides a constant delivery pressure for calibration gases.

Frozen Carbon Dioxide (Dry Ice)

Use of dry ice for addition of CO_2 to chambers is not recommended because of the difficulties in obtaining a uniform release of CO_2 and the high cost of handling this CO_2 source.

Salt with Acid

Production of CO_2 from salts such as sodium carbonate or potassium carbonate with the addition of acid is useful for the addition of radioactive CO_2 to small chambers for labeling studies, but this practice is not recommended for regular use in controlled environment chambers because of the complexities of maintaining the salt-acid system.

Terminology and Units

Carbon dioxide concentration has traditionally been reported in the United States as parts per million (ppm) and in the United Kingdom and Europe as volume per million (vpm or ppmv), but a wide range of units has been used, making comparison between studies difficult (Krizek, 1979). During the past 20 years, there has been a growing trend in scientific literature to adhere to the International System of Units (SI). Since the mole is the SI unit for concentration, the currently recommended unit for CO_2 concentration is µmol mol^{-1} instead of ppm. Because the absolute values in µmol mol^{-1} are the same as those for µL L^{-1} or ppm on a volume basis, there is no need for interconversions.

Carbon dioxide concentration, as measured by the infrared gas analyzer (IRGA), will vary with both temperature and pressure. To make accurate comparisons of studies conducted at different times and at different altitudes, CO_2 concentrations should be corrected and reported at standard temperature and pressure (STP). Correction can be made by applying the equation for the Boyle-Charles law:

$$C_c = C_m * \frac{P}{101{,}325} * \frac{273.15}{T}$$

where C_c is the corrected concentration, C_m the measured concentration, T the measured temperature in kelvin, and P the measured pressure in pascals (Pa).

By specifying the CO_2 concentration at STP, one avoids the confusion of having the concentration of the standard gas vary with temperature and pressure. The problem of having the IRGA record CO_2 concentration at ambient temperature and pressure is partly overcome by the fact that it is a calibrated measurement and the calibration gas is specified at STP.

A final problem in expressing CO_2 concentrations is that CO_2 measurements made with an infrared gas analyzer are measured as molecules (or mass) per unit volume whereas the CO_2 standards themselves are formulated in terms of volume of CO_2 per unit volume of dry air or nitrogen. The CO_2 standards will retain this volume ratio at any atmospheric pressure. By definition, using the unit µmol mol^{-1}, which is independent of the mole volume and STP variation effects, bypasses this problem. For maximum accuracy, however, corrections for STP should be made, especially when measurement conditions differ greatly from STP (Krizek, 1979).

Measurement

Infrared Gas Analyzers

Principle of operation. For many years, nondispersive infared (NDIR) gas analysis has been the method of choice for measuring CO_2 concentrations (Bailey et al., 1970; Beckman, 1967; Jarvis and Sandford, 1985; Šesták et al., 1971). For more detailed information on the use and calibration of this type of CO_2 analyzer, see Jarvis and Sandford (1985). The widespread use of this method for scientific purposes is because NDIR analysis instruments offer higher short-term repeatability of measurements as well as greater accuracy than other commercially available methods. At normal atmospheric CO_2 concentrations (350 µmol mol^{-1}), infrared gas analyzers offer a short-term repeatability of 0.2-10 µmol mol^{-1}, depending on the model. With cor-

rect calibration, the NDIR analysis technique offers an accuracy of < 3 μmol mol^{-1} at 350 μmol mol^{-1} in indicating the true CO_2 concentration. The accuracy, however, is only as good as the calibration gases and the degree to which the analyzer response matches the calibration curve supplied by the manufacturer. As discussed, deviations from STP may also influence the calibration. These units are also sensitive to water vapor, drifts with time, and changes in temperature. Manufacturers provide data on the sensitivity of their units to these and other factors, and most current models are much improved over those available in the 1960s and 1970s in this respect.

The ability of this type of analyzer to measure CO_2 is based on the absorption of energy in the infrared region of the electromagnetic spectrum by CO_2 molecules. Nondispersive infrared analyzers compare the CO_2 absorption of infrared radiation in two gas sampling cells. One of these gas sampling units is called the reference cell. A gas of known CO_2 concentration is passed through or sealed into the reference cell, while a gas of unknown CO_2 is simultaneously passed

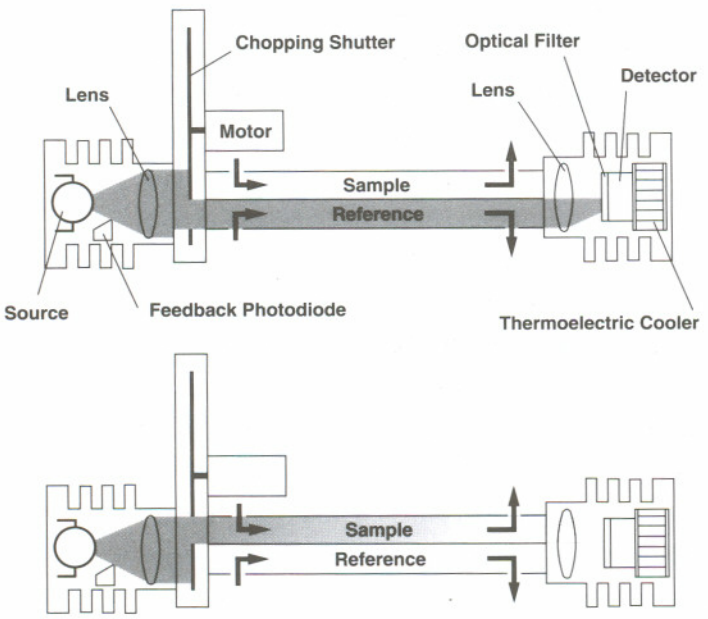

Figure 2. Diagram of a typical nondispersive infrared gas analyzer (provided by LI-COR Inc., Lincoln, Nebraska).

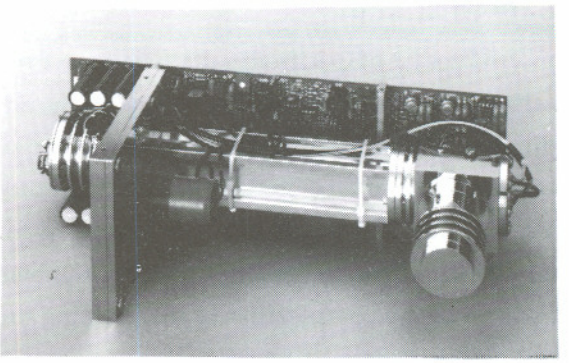

Figure 3. Inside view of a modern nondispersive infrared gas analyzer, showing tubing and electronics used for CO_2 measurement (provided by LI-COR Inc., Lincoln, Nebraska).

through the paired sample cell. Infrared radiation is alternately transmitted through each cell path, and the output of the analyzer is proportional to the difference in absorption between the two cells. Figures 2 and 3 present a diagram and photograph of a typical modern NDIR unit.

Components. Nearly all NDIR analyzers include the following components (illustrated in Fig. 3): the infrared radiation source, a spinning shutter disk or chopper, the reference and sample cells, a CO_2 detector, and an optical filter. The source is a heated filament. The chopper rotates at a high frequency between the source and the sample and reference cells, passing gas alternately between the reference and sample cells. This modulates and stabilizes the output signal and increases sensitivity to the signal. The sample and reference cells will vary in length. Older models required long cells to achieve high resolution, but modern units can achieve equal or greater resolution with quite short path lengths. The interior of the cells may be gold-plated to enhance infrared reflection and to resist tarnishing over time. The detector in modern units is likely to be a solid state lead selenide device and to be thermally stabilized to maintain a constant detector sensitivity. The final component in most modern NDIR analyzers is an optical filter, which tunes the CO_2 detector to the 4.26 micron absorption band for CO_2 and reduces interference from other infrared-

absorbing gasses, such as water. Recently, manufacturers have developed NDIR analyzers that can analyze CO_2 and water vapor simultaneously in the same air stream. This allows simultaneous monitoring of chamber relative humidity. In units with this capability, a dichroic beam splitter at the end of the optical path separates the incoming beam into two parts; one of which goes to a detector filtered to detect radiation absorption by CO_2, but not water, and the other configured to detect water, but not CO_2 (Fig. 4).

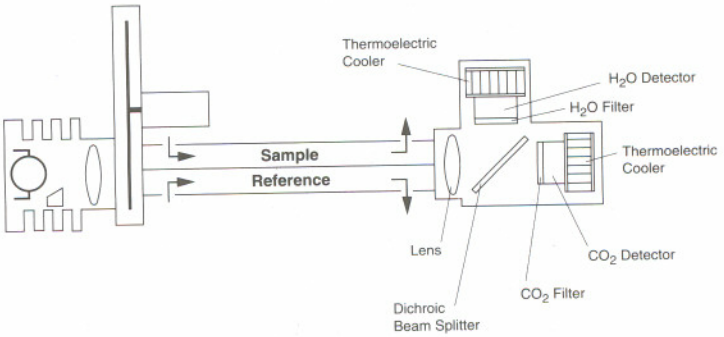

Figure 4. Diagram of a detector featuring a semiconductor photodetector and closed optical filters (provided by LI-COR Inc., Lincoln, Nebraska).

Mode of operation. Although all NDIR analyzers have a reference and a sample cell, the cells may be configured in two different modes: absolute or differential. Most NDIR analyzers are designed to operate accurately in only one of these two modes, but some manufacturers have designed their machines to operate in either mode. The LI-COR machines are basically differential analyzers that can also be operated in the absolute mode. In absolute mode, the reference cell is maintained at a CO_2 concentration of 0 either by chemically "scrubbing" incoming CO_2 or by providing a CO_2-free reference gas. The gas whose concentration is to be measured is passed through the sample cell. In an instrument to be operated only in the absolute mode, the reference cell is sealed with a CO_2-free and water-free gas inside. If an analyzer is to be used only for monitoring CO_2 concentrations in growth chambers, it is more practical to use an absolute mode instrument because no scrubbing of the reference gas is necessary and no reference gases need to be purchased beyond those required for calibration.

If the analyzer is to be used for photosynthetic measurements as well as chamber monitoring, the choice between the two types of analyses is more difficult. In the absolute mode, NDIR analyzers can be used to make transient measurements of photosynthesis by sealing a plant in a closed chamber and monitoring the decline in CO_2 concentrations from photosynthetic CO_2 uptake. Differential analyzers have been used more frequently than absolute analyzers, however, to measure plant photosynthesis and respiration. In this system, the plant is enclosed in an unsealed chamber. Typically, in this type of system, ambient air is passed through the reference cell of the NDIR analyzer before it is provided to the plant. The CO_2 concentration of this incoming air is compared with that of the airstream exiting from the leaf chamber, which is passed through the sample cell. Because CO_2 is not progressively depleted in the chamber, this is considered to be a steady-state system. In this type of system, CO_2 uptake can be monitored while the plant is exposed to a range of temperatures, irradiances, or CO_2 levels.

In deciding whether to use an absolute or differential mode system, calibration range is the most important factor to consider. Most differential analyzers can be used in the absolute mode by comparing the sample with a CO_2-free airstream, but the calibration range may be too low to monitor chamber CO_2 concentrations. If, for example, the analyzer can only be calibrated from 0 to 50 $\mu mol\ mol^{-1}$, a typical range for differential analyzers used for steady state photosynthetic measurements, it will not be accurate in monitoring at ambient chamber CO_2 concentrations of 300-400 $\mu mol\ mol^{-1}$. Similarly, an ab-

solute analyzer may not measure accurately at very low CO_2 concentrations if it is designed to be calibrated at ambient CO_2 or higher, reducing its effectiveness at the low CO_2 concentrations. Older analyzers were only accurate within a narrow range of CO_2 concentrations above and below that at which they were calibrated. Newer analyzers are much improved in this respect, but the range in which the analyzer will be used should still be matched as closely as possible with the midpoint of the instrument's calibration range.

Calibration. Problems in using CO_2 analyzers most frequently arise with CO_2- and water-permeable gas lines on the airstream to be analyzed and inadequate calibration. These and other problems are discussed in detail in Jarvis and Sandford (1985) and Šesták et al. (1971). Cylinders of "standard" carbon dioxide mixtures obtained from gas suppliers should be further checked against tanks with known concentrations even though stated by the supplier to be calibrated. For example, Bate et al. (1969) showed that suppliers' specifications for gas components of less than 1% of the total mixture, such as CO_2, were usually only within ± 5% of the concentration specified when checked against National Institute of Standards and Technology (NIST) gas mixtures (1% tolerance at the 99% confidence level). Table 2 illustrates examples of departures in CO_2 concentration between the suppliers analysis and that of Bate et al. (1969). To calibrate your CO_2 gas cylinders, high accuracy CO_2 standards (in air) having a total uncertainty (95% confidence level) not exceeding 0.1% may be purchased as Standard Reference Materials in 0.85 and 4.25 m^3 cylinders from the National Institute of Standards and Technology (Gaithersburg, Maryland) (Zielinski et al., 1986). Another option is to send a sample of your CO_2 gas to a laboratory such as Battelle Inc. (Cincinnati, Ohio) or the Scripps Institute of Oceanography (San Diego, California). Scientists in these laboratories will analyze your CO_2 calibration gases for a set charge.

When pressure in the calibration tank falls below one-fourth of the initial value, recalibration of the cylinder is necessary. Kelley and Coyne (1972) suggest that cylinder pressures should never be allowed to fall below 3.4 MPa (500 psi), not only because of concentration changes, but also because insufficient gas may preclude accurate analysis of the remaining CO_2 in the cylinder. In addition, when calibrating and operating infrared analyzers, the flow of CO_2 through the sample and reference cells should be within the range specified by the manufacturer (usually 0.2-2.0 L min^{-1}). A high flow rate, such as might be caused by a sudden release of regulator pressure on a tank of calibration gas, not only will result in inaccurate measurement, but also can seriously damage the diaphragms in the analyzer.

Table 2. Comparative analyses of CO_2-air mixtures (μmol mol^{-1}) obtained from commercial suppliers (adapted from Bate et al., 1969).

Stated concentration μmol mol^{-1} (A)	Suppliers' analysis μmol mol^{-1} (B)	Bate et al. analysis μmol mol^{-1} (B – A)	Difference in concentration μmol mol^{-1}
250	255	259	+4
50-55	55	62	+7
95-100	98	113	+15
100	110	114	+4
350	348	362	+14
250-260	255	273	+18
360	355	369	+14
340	345	360	+15
290	285	293	+8
340	340	349	+9

Conductimetric

Conductimetric instruments are potentially useful, but their control is generally less precise than that of infrared instruments and they require more routine maintenance (Acock et al., 1977; Bowman, 1968; Clark et al., 1941; James, 1969). Conductimetric (electrochemical) methods involve the measurement of electrical con-

ductivity of CO_2 dissolved in distilled water. In conductimetric systems, air is bubbled through deionized water where some of the CO_2 dissolves to form carbonic acid, which is passed into a conductivity cell and the resistance measured electrically. The higher the CO_2 concentration, the lower the resistance. The water then flows back to a deionizing column before returning to the bubbling chamber, providing a closed loop for continuous measurement.

Kimball and Mitchell (1979) described a modification of Slack and Calvert's (1972) system including several improvements, the most important of which is temperature compensation. The reported range of their instrument is 0-3000 µmol mol^{-1} CO_2. Their system can be built for a relatively low cost and has been used successfully for continuous monitoring of CO_2 concentrations in the greenhouse (Willits and Peet, 1989). Calibration is required, as with any system, with the frequency depending on the desired accuracy of control and the concentration range covered. When calibrated weekly, the conductimetric system for greenhouse monitoring and control described by Willits and Peet (1989) was accurate within 10% in maintaining greenhouse CO_2 concentration at 1000 µmol mol^{-1}.

Photochemical

The least expensive and simplest method of measuring CO_2 concentration involves the use of comparative colorimetry (Slavik and Čatský, 1965); colorimetry, however, can only provide precision to 50 to 100 µmol mol^{-1} and is useful for spot, rather than continuous, measurement. Commercially available chemical gas detectors (Hanan, 1984) can be purchased from most laboratory supply houses and greenhouse equipment suppliers. The chemical is usually contained in a glass tube and changes color as an air sample is drawn through the tube. A new tube must be used for each determination.

Various colorimetric procedures have also been described by Sharp (1964), and a portable system is described by Enoch et al. (1970). We do not recommend these systems for growth chambers, however, because they are not adapted to precise and continuous monitoring.

Monitoring and Control

Continuous monitoring and control of CO_2 levels in chambers is difficult and usually expensive. Infrared gas analyzers frequently are used for continuous monitoring, and because of their cost, they are often connected to computerized systems for controlling several environmental chambers (Fabreguettes et al., 1992; Kimball, 1990; McFarlane and Pfleeger, 1985; Roy and Jones, 1988). A microcomputer is frequently used to compare the measured CO_2 concentration for each unit with its programmed level. A diagram of the control system used by Fabreguettes et al. (1992) is shown in Fig. 5. They also describe the air sampling layout and the monitoring program. With this system, a CO_2 concentration from 50 to 10,000 µmol mol^{-1} can

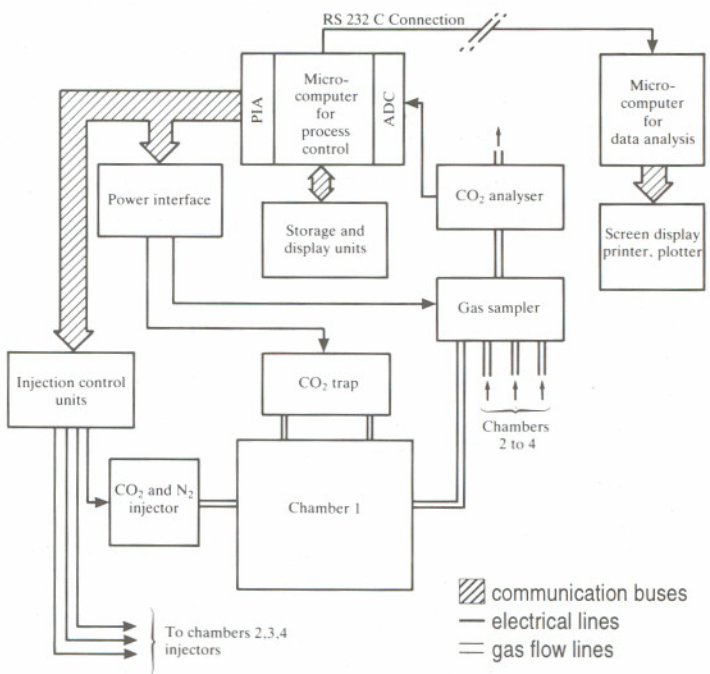

Figure 5. Diagram of CO_2 monitoring and control system for four controlled environment chambers (Fabreguettes et al., 1992).

be maintained independently in four growth chambers. Air samples taken every 12 minutes in the chambers allow calculation of plant photosynthetic rates. These calculations were used to determine the injection frequency for pure CO_2 necessary to replace that taken up by the plants.

In the Duke University Phytotron, the current CO_2 value for each chamber is compared with the previously sampled value to predict the next value (Hellmers and Giles, 1979). If the new value is far from that programmed, the number of seconds of injection per minute is adjusted accordingly. Thus large swings in CO_2 are quickly damped out by the control program once the source of the disturbance is removed. After each sampling of a set of chambers, the IRGA is calibrated by the microcomputer using a calibration gas. The injection rate and CO_2 level of each chamber are stored on a floppy disk. A set concentration of 350 µmol mol^{-1} can be maintained within ± 10 µmol mol^{-1}, which represents a ± 3% variation. This same percentage variation can be maintained at higher concentrations (L. Giles, personal communication).

Maintaining CO_2 levels below ambient levels is much more difficult because the air must be scrubbed by absorption on columns or in alkaline solutions such as soda lime. Considerable equipment and the handling of corrosive materials are required for scrubbing. Pallas (1986) describes a system in which populations of rapidly growing C4 plants such as corn, sorghum, or millet are grown in a chamber to reduce the CO_2 level or in a paired chamber with which air is exchanged. A scrubbing system that uses moistened filters has also been implemented at the Duke University Phytotron. When the control computer indicates excessive CO_2 levels, chamber air is pulled through a 10 cm screened tray containing moistened vermiculite and hydrated lime (5:1 v:v). Chamber CO_2 concentration can be reduced to 270 µmol mol^{-1} with daily changes of the trays (L. Giles, personal communication).

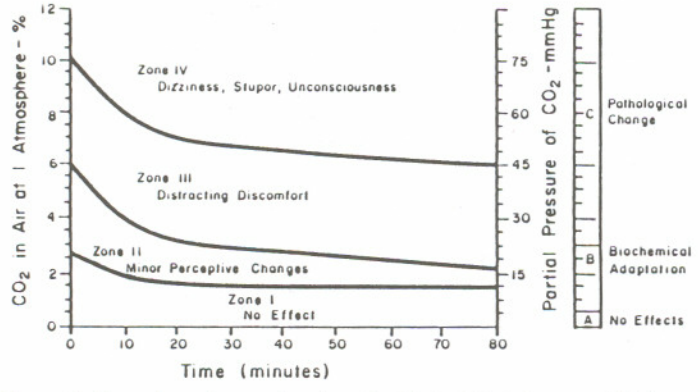

Figure 6. Time dependence of carbon dioxide toxicity to humans (Glatte and Welch, 1967).

Safety

Generally, CO_2 levels used in controlled environment chambers do not pose a safety risk (Fig. 6). There is certainly a possibility that an undetected malfunction of a solenoid valve could result in an acute situation where continuous injection increases CO_2 up to levels where it is toxic to humans. Human toxicity from CO_2 is about 100,000 µmol mol^{-1} (10% of air volume), 100 times higher than the usual level for CO_2 enrichment. Above 50,000 µmol mol^{-1} (5%), however, dizziness and loss of consciousness can result (Glatte and Welch, 1967; Roth, 1964). Prolonged exposure above 20,000 µmol mol^{-1} (2%) should also be avoided, although no detrimental effects should result from short exposures. A low-maintenance gas monitor can be purchased to warn of potentially hazardous CO_2 levels. The ADC 2000 continually monitors CO_2 and an alarm is activated when concentrations exceed preset levels. Carbon dioxide levels can be monitored either from 0 to 2% or from 0 to 0.3%. In the United States, the distributor is CEA Instruments, Inc., Emerson, New Jersey.

Plants are more sensitive than humans to chronic exposure to high CO_2 levels. Tomato plants, for example, show foliar inrolling with

chlorosis and a purple pigmentation at 1000 μmol mol^{-1} (0.1%) CO_2 (Tripp et al., 1991). Thus investigators may be alerted to chronic overexposure to CO_2 by plant response even if monitoring equipment does not detect the problem.

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