

CARBON DIOXIDE WITHIN CONTROLLED ENVIRONMENTS; THE COMMONLY NEGLECTED VARIABLE

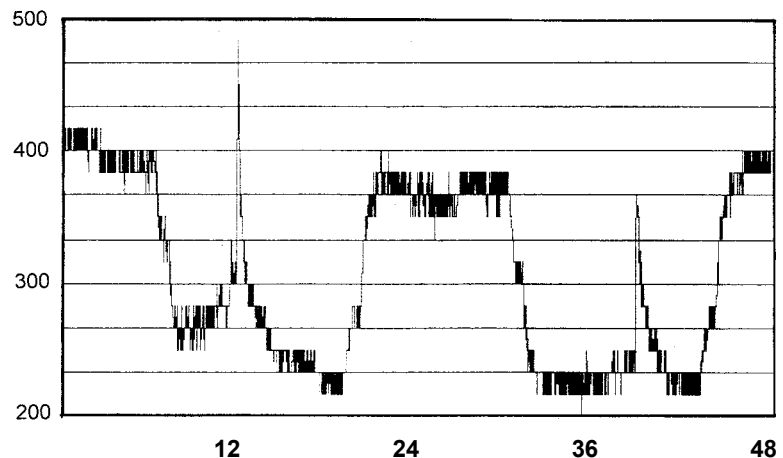
Mark Romer

McGill University Phytotron, 1205 Dr Penfield Avenue, Montreal, Quebec, Canada H3A 1B1
(mark.romer@mcgill.ca)

Despite great improvements in measurement and control technology for growth chambers, some environmental variables continue to be overlooked by the majority of chamber users. It has been suggested that CO₂ is perhaps the least controlled variable within plant growth chambers (Klueter, 1979; Peet and Krizek, 1997). Historically, the decision to incorporate CO₂ measurement and control equipment in growth chambers was influenced by factors including limited commercial availability, high purchase cost, large unit size and considerable maintenance requirements. Typically, only large facilities that specialized in global change research that were equipped with centralized analysis and distribution systems offered continuous, whole chamber CO₂ control to their research users. More commonly, ecologists and physiologists would collect periodic samples of chamber CO₂ levels in association with physiological measurements of their research plants. Over the past decade, CO₂ sensors have become more compact and affordable and are now offered as standard options by commercial growth chamber manufacturers. Despite these improvements, a survey by the author of major controlled environment facilities and equipment suppliers in Australia, Europe and North America in 2001 revealed that fewer than 15% of chambers operating on these continents had CO₂ monitoring equipment installed!

Significant fluctuations in diurnal CO₂ concentrations occur within most growth chambers and have been well documented (Bernier, Stewart and Hogan, 1994). The magnitude of fluctuation varies between chambers and over time depending on chamber and facility design factors, degree of human activity and biomass and physiological characteristics of research plants. Figure 1 illustrates a typical course of diurnal CO₂ concentrations in an uncontrolled growth chamber planted with *Brassica napus*. Note the significant drawdown of CO₂ concentration during the light phase

Figure 1: CO₂ concentration ($\mu\text{mol mol}^{-1}$) measured over 48 hours in a 7 m³ growth chamber without additive or scrubbing control systems.



and mid-day spikes associated with human entry into the chamber. Two component control systems, (additive and scrubbing) are required to eliminate CO₂ fluctuations and provide good environmental control of this variable. Both systems require the use of a dedicated infrared gas analyzer or comparable CO₂ measurement device. Guidelines concerning instrumentation,

measurement and reporting of CO₂ within growth chambers may be found in Tibbitts, Sager and Krizek (2000).

Additive Systems

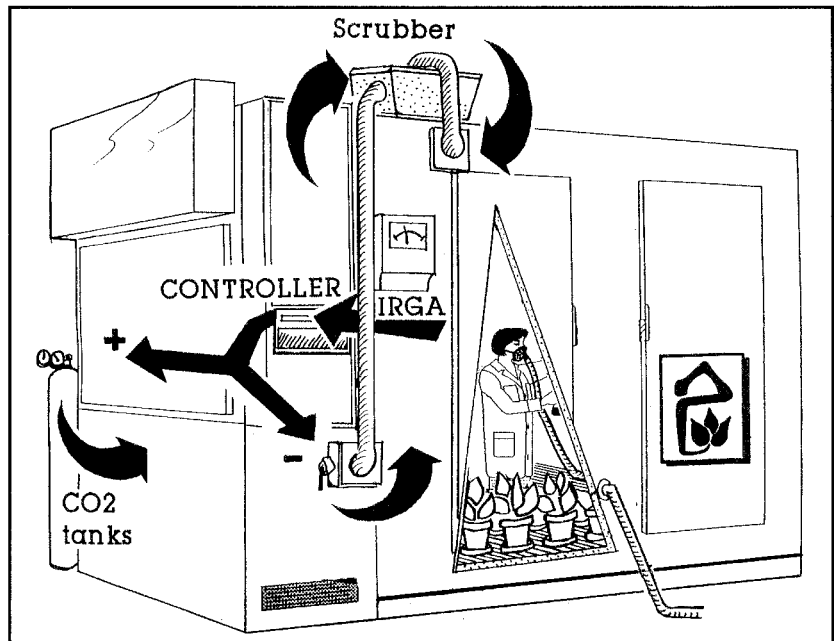
The addition of an additive CO₂ control system to a growth chamber effectively eliminates the daytime drawdown of CO₂ levels by plant photosynthetic activity and permits the programming of elevated CO₂ concentrations in the range between 500 to 3000 $\mu\text{mol mol}^{-1}$. The components of a typical system include one or two bottled gas cylinders (bone-dry or commercial grade), two-stage pressure regulating valves, pressure reduction valve and a dosing solenoid. At the McGill Phytotron, a two-tank system is used with tank regulators adjusted differentially to 70 and 100 kPa respectively. By using differential output pressures, the 100-kPa tank is drained before the 70-kPa tank and the chamber does not run out of gas unexpectedly. Most commercial chamber manufacturers now offer additive CO₂ control systems as options within their product lines.

Scrubbing Systems

The challenge of scrubbing CO₂ from growth chamber air is greater and systems tend to homemade designs rather than standardized commercial equipment. Some commercial suppliers have provided exhaust dampers to expel chamber air when CO₂ levels rise above programmed setpoints. This option successfully reduces nighttime CO₂ buildup within chambers but does not provide an accurate control of daytime CO₂ concentrations within the chamber. This option may also contribute to elevating ambient CO₂ levels within larger facilities equipped with many chambers.

At the McGill Phytotron, a scrubber system was designed in 1990 modeled on a system initially developed at the Duke University Phytotron (Fig. 2). The system bypasses a stream of chamber air through an exterior container filled with scrubbing medium. The system uses the existing air exchange ports and profits from the natural air velocity of the growth chamber's blowers. A 10 litre plastic container is fitted with plastic, wire-reinforced tubing (7.5 cm diameter) and is filled with 4 litres of scrubbing medium. The inlet port of the container is fitted with a fine mesh screen (2-mm mesh) and the

Figure 2: Graphic depiction of additive and scrubbing systems utilized in the McGill Phytotron. Note researcher wearing gas mask connected to exterior air venting system.



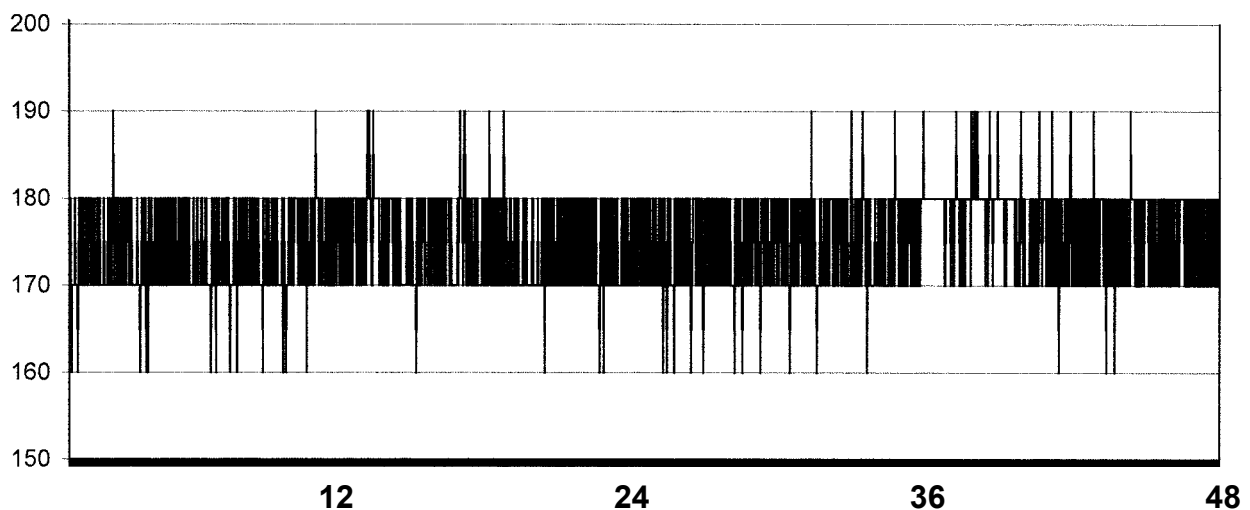
lid is made airtight with foam weather-stripping and pressure clips. Airflow through the scrubber may be continuous or controlled by means of a damper motor installed at the chamber exhaust port. The addition of such a motor prevents unnecessary scrubbing of chamber air and lowers operating costs. Two different scrubbing products have been successfully utilized.

- Sodasorb CO₂ adsorbent medium (W.R. Grace, Atlanta, Georgia, (800)-438-7632) is a white, granular product that includes an ethyl violet indicator that assists users in determining when the product needs to be replaced. Sodasorb is relatively expensive (Table 1), but requires less maintenance, typically lasting 1-2 weeks between refills. The replacement rate is dependent on the volume of air being scrubbed, quantity of plant biomass, plant gas exchange rates and environmental conditions. Also available is Soda Lime CO₂ adsorbent (Mallinkrodt Baker, Phillipsburg, New Jersey (800) 582-2537).
- Hydrated lime Ca(OH)₂ (Graymont Dolime, Genoa, Ohio (800) 537-4489) is much less expensive and may be mixed with moist vermiculite to produce an equally effective alternative. Hydrated lime is however, extremely caustic in nature, more difficult to handle and must be changed daily to achieve good CO₂ control.

Results

Over the past decade we have used our CO₂ control systems for both static and dynamic experiments with considerable success. Typical variation is less than 3% or +/- 10 $\mu\text{mol mol}^{-1}$ from programmed setpoints. Statistical analysis of a 6 month, 4 chamber dynamic forest ecology study conducted at setpoints of 350 and 700 $\mu\text{mol mol}^{-1}$ found CO₂ levels remained within 10 $\mu\text{mol mol}^{-1}$ 90% of the time (Wang, Z.M., M.J. Lechowicz and C. Potvin, 1994). Attempts to maintain sub-ambient control setpoints have been equally successful. CO₂ concentrations of 250 $\mu\text{mol mol}^{-1}$ were achieved in larger 7 m³ chambers and levels of 170 $\mu\text{mol mol}^{-1}$ were recently attained in smaller 2 m³ chambers. (Fig. 3).

Figure 3: CO₂ concentration measured over 48 hours in a 2 m³ growth chamber (setpoint = 170 $\mu\text{mol mol}^{-1}$, mean = 174.9 $\mu\text{mol mol}^{-1}$, SD = 5.5, n = 2880)

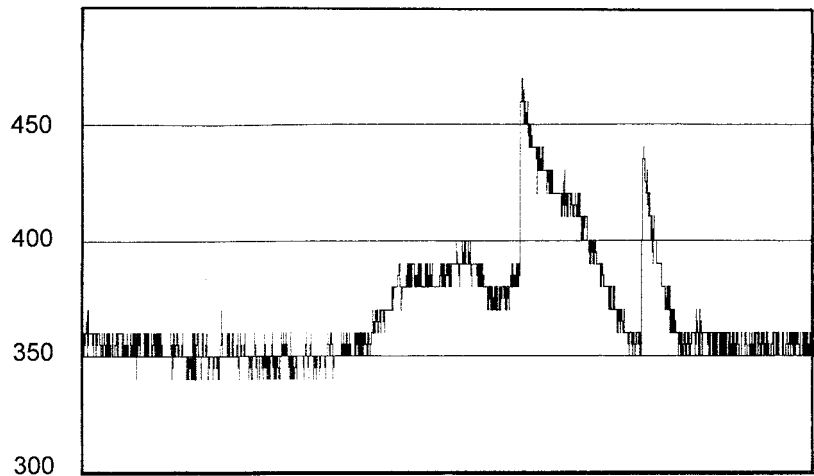


Several factors limit the effectiveness of these CO₂ control systems. The control system is particularly susceptible to human disturbances at setpoints below 500 μmol mol⁻¹. Chamber CO₂ concentrations typically require 2-3 hours to return to control set points after human entry into the growth chamber (Fig. 4). The impact of human intervention may be lessened by utilizing a gas mask connected to an exterior ventilation system (Fig. 2), by use of automatic irrigation systems and by limiting chamber access.

The control system also has difficulty controlling CO₂ levels during periods of high ambient CO₂ within the facility. Elevated CO₂ is particularly noticeable in urban centres and can be attributed to seasonal effects, atmospheric inversions and increased human activity. Some improvement may be obtained by increased sealing of the chamber doors and walls and minimizing entry into the chamber during periods of elevated ambient CO₂.

There is an increased risk of air contaminant accumulation in CO₂ control chambers owing to the greatly reduced air exchange rate with the exterior environment. Several contaminants including C₂H₄, H₂S, SO₂, CO and NH₃ have been reported as byproducts of commercial-grade CO₂ production (Peet and Krizek, 1997). Other contaminants may be derived from chamber construction compounds or research materials (Tibbitts, 1997).

Figure 4: Impact on CO₂ concentration (μmol mol⁻¹) of human activity within a growth chamber (10 hour period)



The cost of implementing CO₂ control may be divided into initial and monthly maintenance components. The only significant initial cost is the purchase of a suitable sensor. Currently, reliable and compact gas analyzers are available in the range of \$ 400 - \$ 1,200 US. The cost of integrating a sensor into the chamber's existing control system will vary and must be discussed with the manufacturer directly. Other initial equipment costs including regulators, valves and solenoids will cost between \$ 300-500 US.

Table 1: Cost of maintaining CO₂ control (range 200-350 μmol mol⁻¹) using different adsorbent media. (\$ US / m³ chamber volume / month)

Components	Biomass factor	
	Low	High
Calibration gases (IRGA)	\$ 0.50	\$ 0.50
CO ₂ gas (bone-dry or commercial grade)	\$ 0.80	\$ 1.20
Sodasorb adsorbent medium (W.R. Grace)	\$ 20.00	\$ 30.00
Hydrated lime and vermiculite	\$ 6.00	\$ 9.00

The monthly maintenance cost of implementing CO₂ control varies greatly depending on the concentration of CO₂ desired, adsorbent medium used, chamber volume, plant biomass and ambient CO₂ levels in the surrounding facility. At control levels below 350 μmol mol⁻¹, costs were found to vary between \$ 21.30 and \$ 31.70 m⁻³ month⁻¹) for Sodasorb brand adsorbent medium (Table 1). Material costs for hydrated lime and vermiculite were 70% lower, but labour requirements were much higher owing to the fact that adsorbent media needed to be changed daily. Monthly costs dropped by at least 50% at CO₂ setpoints above 400 μmol mol⁻¹. At concentrations over 500 μmol mol⁻¹, scrubbing medium was rarely required to achieve good control in growth chambers.

Conclusion

Now that CO₂ can be precisely and inexpensively controlled within growth chambers, it would seem that routine measurement, control and reporting of this important environmental variable should be more universally implemented in controlled environment studies.

In order to accomplish this, three steps are required:

- (1) More growth chambers need to be equipped with CO₂ monitoring and control capabilities,
- (2) Research users must be encouraged to monitor actual CO₂ conditions within their chambers and incorporate this variable in their experimental designs and analyses and
- (3) More research is required by commercial manufacturers in the development of standardized CO₂ scrubbers for commercial growth chambers.

References

- Bernier, P.Y., Stewart, J.D. and Hogan, G.D. (1994) Quantifying the uncontrolled CO₂ dynamics of growth chambers. *Journal of Experimental Botany* 45, 1143-1146.
- Klueter, H.H. (1979) Carbon Dioxide: Critique II. In: *Controlled Environment Guidelines for Plant Research*. Tibbitts, T.W. and Kozlowski, T.T. (eds.), Academic Press, New York. pp. 235-240.
- Peet, M.M. and Krizek, D.T. (1997) Carbon Dioxide. In: *Plant Growth Chamber Handbook*. Langhans R.W. and Tibbitts, T.W. (eds.), North Central Regional Research Publication No. 340, Iowa State Agr. & Home Econ. Expt. Stat. Rpt. No. 99, Ames. pp. 65-79.
- Tibbitts, T.W., Sager, J.C. and Krizek, D.T. (2000) Guidelines for Measuring and Reporting Environmental Parameters in Growth Chambers. *Biotronics* 29, 9-16.
- Tibbitts, T.W. (1997) Air Contaminants. In: *Plant Growth Chamber Handbook*, Langhans R.W. and Tibbitts, T.W. (eds.), North Central Regional Research Publication No. 340, Iowa State Agr. & Home Econ. Expt. Stat. Rpt. No. 99, Ames. pp. 81-86.
- Wang, Z.M., Lechowicz, M.J. and Potvin, C. (1994) Early selection of black spruce seedlings and global change: which genotypes should we favor? *Ecological Applications* 4, 604-616.