BRIEF COMMUNICATION

Chamber response time: a neglected issue in gas exchange measurements

I. WEISS*, Y. MIZRAHI*, and E. RAVEH**,+

*Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel**
*Gilat Research Center, mobile Post Negev, 85280, Agricultural Research Organization, Ministry of Agriculture, Israel***

Abstract

When the dimensions of standard commercial chambers for measuring gas exchange cannot accommodate the object being measured, scientists construct their own chambers. The time needed to reach chamber steady state (chamber response time) depends on net system volume (*e.g*. chamber and tubing volume) and airflow. Unfortunately, some authors take chamber response time into consideration while others ignore it. We present the formula for calculating chamber response time.

Additional key words: chamber steady state; *Hylocereus*; infrared gas analyser; laboratory-made chambers; net CO_2 uptake; open system; self-clamping chambers.

Widely used in biological and physiological studies, gas exchange measurements (*e.g*. CO_2, O_2, O_3, CH_4 exchange) can focus on specific plant organs—leaf, root, stem, branch, or fruit—or on the entire canopy (Long *et al*. 1996, Burkart *et al*. 2007). In soil and water measurements, small points can undergo gas exchange analyses on whole-ecosystem scales (Edwards and Riggs 2003, Repo *et al*. 2007). With the exception of eddy-covariance, measurements are performed with the chamber attached to or enclosing the measured object. In commercial chambers, the time needed to reach steady state (chamber equilibrations, chamber response time) is well defined. However, the chamber response time in home-made chambers is often ignored.

Gas exchange errors are usually described as being associated with oscillations in condensation of water, pressure drops within the chamber, leaks, edge effects, and reactions of the gas with the chamber walls and tubing materials (Lund *et al*. 1999, Altimir *et al*. 2002, Pons and Welschen 2002, Long and Bernacchi 2003, Jahnke and Pieruschka 2006, Flexas *et al*. 2007, Rodeghiero *et al*. 2007). We address the errors in gas exchange measurements that result from neglecting the chamber response time.

Scientists sometimes build their own chambers to, for example, measure massive plant organs or large soil or water surfaces. These chambers can be either attached to the surface of the measured object or they can enclose it (Raveh *et al*. 1995, Graham and Nobel 1996, Liang *et al*. 2003, Nobel and De La Barrera 2004, Ben-Asher *et al*. 2006, Repo *et al*. 2007). Investigators occasionally design their chambers to be used in combination with commercial gas exchange systems (*LI-6400 LI-COR*, Lincoln, NE, USA; *CIRAS-II*, *PP Systems*, Hitchin, UK; *LCA4*, *ADC Biosciences*, Hoddesdon, UK; *PM48 PhyTech*, Rehovot, Israel). While some companies build their chambers to accommodate laboratory-made chambers as add-ons to their infrared gas analyzer (IRGA; *e.g*. *LI-COR*), describing it in detail as part of their measurement protocol (*e.g*. chamber size, shape, and airflow), other companies do not offer this option (*PM 48 PhyTech*). Without the use of laboratory-made chambers, however, we would not have any information on, among other subjects, succulent Crassulacean Acid Metabolism (CAM) CO_2 uptake and inflorescence gas exchange (Tissue and Nobel 1990, Ben-Asher *et al*. 2006).

Received 6 May 2008, *accepted* 26 August 2008.
+Corresponding author; fax: +972-8-9926485, e-mail: eran@agri.gov.il
Abbreviations: CAM − Crassulacean Acid Metabolism; IRGA − infrared gas analyzer.
Acknowledgements: We thank Mr. Patrick Martin for styling the manuscript.

Open system gas exchange measurements should be taken only after the gas reaches a steady state (*i.e.* becomes stable), the time required for which is characterized by an exponential pattern [Fig. 1*A*, *LI-COR* (2003)]:

$$C_t = C_{in} - (C_{in} - C_{out})\, e^{-(f t\, Vs-1)} \quad (1)$$

where C_t is the chamber gas concentration at time t, C_{in} and C_{out} are the gas concentrations in the inlet and outlet flows, respectively [μmol mol^{-1}], f is the air flow rate [cm^3 s^{-1}], t is the time [s], and V_s is the system air volume [cm^3] which should be calculated as follows:

$$V_s = V_c + V_t + V_i - V_e \quad (2)$$

where V_c is the chamber volume, V_t is the tubing volume, and V_i is the IRGA chamber volume. V_e is the volume of the object enclosed within the chamber, thus reducing system volume: for example, the organ being measured (*e.g.* shoot, fruit, vegetation, and soil), snowfall that enters the chamber, and the fans and other pneumatic elements all affect chamber volume. Soil measurement has the same effect on chamber volume when soil is inserted into the chamber. From Eq. 1, the system time constant τ [the time required to obtain 63 % of the final measured gas concentration within the chamber; *LI-COR* (2003)] is therefore:

$$\tau = V_s\, f^{-1} \quad (3)$$

Because the time to steady state is an exponential characteristic, by definition it implies that at three time constants (3 τ) chamber gas concentration should reach 95 % of its steady state. As a result, Eq. 3 shows that chamber response time should only be affected by airflow rate and system volume.

Focusing on articles for which we could calculate the time constant τ, we multiplied τ by three and compared our results to the waiting times used by the authors (Table 1). Some of the articles tested and described their chamber response times in detail and indeed reached steady state. Others were less descriptive regarding chamber response times, but their waiting times were sufficient or above that needed to reach steady state (Lange *et al*. 1997, Burton and Pregitzer 2002, Liang *et al*. 2003, Suh *et al*. 2006). Still other researchers did not consider system volume (V_t, V_e, and V_i), flow rate, or the required calculation of waiting time, and as a result, they started measuring before achieving steady state (Hari *et al*. 1999, Altimir *et al*. 2002, Edwards and Riggs 2003, Wang *et al*. 2003, Ben-Asher *et al*. 2006, Kolari *et al*. 2007, Zha *et al*. 2007).

Finally, some researchers used different chamber volumes (Kolari *et al*. 2007) or airflows (Altimir *et al*. 2002) within the same experiment but without changing the waiting times accordingly. Yet, reducing V_t during the measurements only leads to a shorter τ, and therefore would not harm the accuracy of the measurements; increasing V_t during the measurements, however, leads to a longer τ and subsequent error. Kolari *et al*. (2007), who measured shoot CO_2 exchange at two different study sites, used chambers of 1 000 cm^3 at one site and 3 500 cm^3 at the second site, but in both locations they waited 60 s. That waiting time proved sufficient for the measurements done with the smaller chamber, but for the larger chamber, 60 s was insufficient, equalling about 50 % of the required time. Altimir *et al*. (2002), who monitored gas exchange year-round, used an airflow rate of 83 cm^3 s^{-1} during the summer and 50 cm^3 s^{-1}

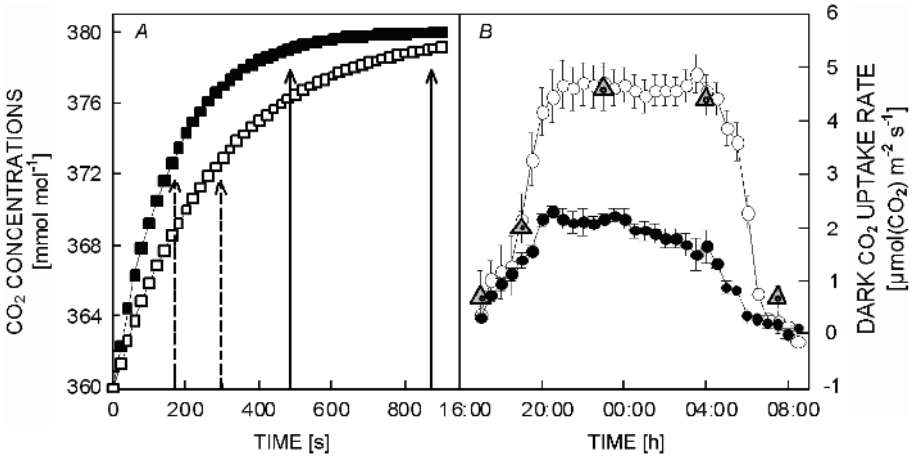

Fig. 1. (*A*) Theoretical exponential change in chamber CO_2 concentration *vs*. time. Initial chamber CO_2 concentration was 360 μmol mol^{-1} and incoming CO_2 concentration was 380 μmol mol^{-1}, V_s = 2 400 cm^3, f = 15.0 cm^3 s^{-1} (*black squares*) or 8.3 cm^3 s^{-1} (*white squares*). *Dashed arrows* indicate τ and *solid arrows* indicate 3 τ (adapted from *LI-COR*, 2003). (*B*) Dark CO_2 uptake in 4 cm^3 and 2 400 cm^3 chambers. Measurements were taken over dark periods every 30 min for *H. undatus* with a 2 400 cm^3 chamber (*black circles*) and with a 4 cm^3 chamber (*white circles*). *Gray triangles* represent measurements taken with a closed system infrared CO_2 analyzer *LI-COR 6200* modified for use on stem surfaces as described by Raveh *et al*. (1995). Measurements were taken over a period of 5 d (14−18 February, 2008); average day/night air temperatures and daily PPF were 25/15 °C and 13 mmol(photon) m^{-2} d^{-1}, respectively. Means ± SE (n = 3−8 plants).

Table 1. V_s represents the total system volume (tubing and chamber) [cm^3] reported in each paper. For the papers in which tubing volume was absent, it was not part of V_s calculations. Yet in such cases, ignoring tubing volume only minimized the errors in waiting time. The volume of the object enclosed within the chamber was always ignored, since its contribution to V_s in the present papers was negligible (estimated as less than 5 % of V_s) or non-relevant (*i.e*. when the chamber was attached to the surface of the measured object or when calibrations were done on an empty chamber). f is the airflow of the system reported in each paper. 3 τ is the required waiting time for achieving 95 % of steady state, based on Eq. 3. T is the waiting time used by the authors [s or % of the required waiting time]. *Data obtained from either a reference within the paper or personal communication; in cases with more than one option, we always considered the parameter that would result in the smallest error (*e.g*. the higher flow rate).

Steady state achieved	Target	V_s [cm^2]	f [$cm^3\ s^{-1}$]	3 τ [s]	T [s]	[% of 3 τ]	Reference
yes	shoot	1000	83*	36	60	167	Kolari *et al.* (2007)
	soil	405000	2083	1013	1200	119	Liang *et al*. (2003)
	lichens	190	8	68	200	292	Lange *et al*. (1997)
	calibration	76	3*	91	480	526	Burton and Pregitzer. (2002)
	stem	104	15	21	120	571	This study
no	calibration	3978	17	694	70	10	Hari *et al*. (1999)
	stem	2351*	15	470	120	26	Ben-Ascher *et al*. (2006)
	shoot	3500	50	210	70	33	Altimir *et al*. (2002)
	shoot	2370	13	535	220	41	Wang *et al*. (2003)
	shoot	3500	83	126	70	56	Altimir *et al*. (2002)
	shoot	3500	83*	126	60	48	Kolari *et al.* (2007)
	shoot	3854	25	483	60	13	Zha *et al*. (2007)
	soil	5417	25	650	60	9	Zha *et al*. (2007)
	soil	4712	15	942	720	76	Edwards and Riggs (2003)

throughout the rest of the year, all the while using the same chamber. As a result, they waited only 56 and 33 % of the times required for steady state in the summer and the rest of the year, respectively.

In our experiment, we used mature stems of the vine cacti *Hylocereus undatus* (Haworth) Britton & Rose plant. Measurements were made on the third stem segment from the ground. The plants were grown in Beer-Sheva, Israel, in a controlled temperature greenhouse (average day/night air temperature of 25/15 °C), fertigated with 0.5-strength Hoagland's solution, and were irrigated every second day. Radiation measure ments during the 4 measured days registered about 13 mmol(photon) $m^{-2}\ d^{-1}$. We measured CO_2 uptake using the 2 400 cm^3 chamber and the IRGA (*PM48* with a flow rate of 8.3-15.0 $cm^3\ s^{-1}$ and a waiting time of 120 s) used by Ben-Asher *et al*. (2006) and compared it with the results obtained with the 4 cm^3 lab-made chamber, as was originally designed by the manufacturer. In addition, 5 spot measurements were made with the *LI-COR 6200* portable photosynthesis closed system together with the chamber used by Raveh *et al*. (1995).

Ben-Asher *et al*. (2006) used for the new 2 400 cm^3 chamber the waiting time recommended by the manufacturer (*PhyTech* 2002) for its original 4 cm^3 chamber, *i.e*. 120 s. But the IRGA airflow rate is limited by the manufacturer (*PhyTech* 2002) to between 8.3 and 15.0 $cm^3\ s^{-1}$. Using the original chamber airflow (8.3 to 15.0 $cm^3\ s^{-1}$) and volume (4 cm^3) gave response times of 0.8 to 1.4 s. In contrast, increasing chamber volume to 2 400 cm^3 led to response times of 480 to 867 s (depending on flow rate; Fig. 1*A*), such that the waiting times used by Ben-Asher *et al*. (2006) were 26 to 14 % of those required for steady state, respectively. Measurements of net CO_2 uptake obtained from the 4 cm^3 chamber were in agreement with those obtained with the *LI-COR 6200* closed system. Increasing the chamber volume (from 4 to 2 400 cm^3) while preserving the original chamber waiting time and airflow led to a-50 % decrease in net CO_2 uptake values (Fig. 1*B*), as was hypothesized. One possible reason for ignoring chamber volume by some authors could be because in open systems chamber volume is not taken into account when calculating gas exchange rates. Chamber response time in continuance measurements is a less critical parameter, as in spot measurements and in self-clamping chambers (where the chamber remains open between measurements), since the system is assumed to reach steady state during the course of the experiment.

Variations in gas exchange rates measured by different researchers are usually related to differences in the analytical methods used to measure gas exchange (closed *vs*. open system), in the growth conditions, in genetic drift, plant age, and other physiological factors (Longdoz *et al*. 2000, Lake 2004). We suggest that a miscalculation of the time response also contributes to the variation found in gas exchange measurements. Chamber response time can be altered by changing the airflow or limiting chamber volume. However, increasing the airflow is not always practical due to the limited range of flow rates available in commercial air pumps (*e.g*. *PM48*). Meanwhile, limiting the chamber volume depends on the size of the target object. A third option entails extending the waiting time, but that can induce

high temperatures and humidity in the chamber. Therefore, we advise researchers to estimate chamber response time before the chambers are manufactured, as was done by Lange *et al.* (1997) and Liang *et al.* (2003). In addition, we recommend that time response calculations constitute a standard data requirement in any gas exchange manuscript.

References

Altimir, N., Vesala, T., Keronen, P., Kulmala, M., Hari, P.: Methodology for direct field measurements of ozone flux to foliage with shoot chambers. – Atmos. Environ. **36**: 19-29, 2002.

Ben-Asher, J., Nobel, P.S., Yossov, E., Mizrahi, Y.: Net CO_2 uptake rates for *Hylocereus undatus* and *Selenicereus megalanthus* under field conditions: Drought influence and a novel method for analyzing temperature dependence. – Photosynthetica **44**: 181-186, 2006.

Burkart, S., Manderscheid, R., Weigel, H.J.: Design and performance of a portable gas exchange chamber system for CO_2 and H_2O flux measurements in crop canopies. – Environ. exp. Bot. **61**: 25-34, 2007.

Burton, A.J., Pregitzer, K.S.: Measurement carbon dioxide concentration does not affect root respiration of nine tree species in the field. – Tree Physiol. **22**: 67-72, 2002.

Edwards, N.T., Riggs, J.S.: Automated monitoring of soil respiration: A moving chamber design. − Soil Sci. Soc. Amer. J. **67**: 1266-1271, 2003.

Flexas, J., Diaz-Espejo, A., Berry, J.A., Cifre, J., Galmes, J., Kaidenhoff, R., Medrano, H., Ribas-Carbo, M.: Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. – J. exp. Bot. **58**: 1533-1543, 2007.

Graham, E.A., Nobel, P.S.: Long-term effects of a doubled atmospheric CO_2 concentration on the CAM species *Agave deserti*. – J. exp. Bot. **47**: 61-69, 1996.

Hari, P., Keronen, P., Back, J., Altimir, N., Linkosalo, T., Pohja, T., Kulmala, M., Vesala, T.: An improvement of the method for calibrating measurements of photosynthetic CO_2 flux. − Plant Cell Environ. **22**: 1297-1301, 1999.

Jahnke, S., Pieruschka, R.: Air pressure in clamp-on leaf chambers: a neglected issue in gas exchange measurements. – J. exp. Bot. **57**: 2553-2561, 2006.

Kolari, P., Lappalainen, H.K., Hanninen, H., Hari, P.: Relationship between temperature and the seasonal course of photosynthesis in Scots pine at northern timberline and in southern boreal zone. – Tellus **59B**: 542-552, 2007.

Lake, J.A.: Gas exchange: new challenges with *Arabidopsis*. – New Phytol. **162**: 1-3, 2004.

Lange, O.L., Reichenberger, H., Walz, H.: Continuous monitoring of CO_2 exchange of lichens in the field: Short-term enclosure with an automatically operating cuvette. – Lichenologist **29**: 259-274, 1997.

Liang, N., Inoue, G., Fujinuma, Y.: A multichannel automated chamber system for continuous measurement of forest soil CO_2 efflux. – Tree Physiol. **23**: 825-832, 2003.

LI-COR: Interfacing custom chambers to the *LI-6400* sensor head. – *LI-6400* Application note 3. LI-COR, Lincoln 2003.

Long, S.P., Bernacchi, C.J.: Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. – J. exp. Bot. **54**: 2393-2401, 2003.

Long, S.P., Farage, P.K., Garcia, R.L.: Measurement of leaf and canopy photosynthetic CO_2 exchange in the field. – J. exp. Bot. **47**: 1629-1642, 1996.

Longdoz, B., Yernaux, M., Aubinet, M.: Soil CO_2 efflux measurements in a mixed forest: impact of chamber disturbances, spatial variability and seasonal evolution. – Global Change Biol. **6**: 907-917, 2000.

Lund, C.P., Riley, W.J., Pierce, L.L., Field, C.B.: The effects of chamber pressurization on soil-surface CO_2 flux and the implications for NEE measurements under elevated CO_2. – Global Change Biol. **5**: 269-281, 1999.

Nobel, P.S., De la Barrera, E.: CO_2 uptake by the cultivated hemiepiphytic cactus, *Hylocereus undatus*. – Ann. appl. Biol. **144**: 1-8, 2004.

Phytech: PM-48 photosynthesis monitor, Operating instructions (Version *1.0*) – Phytech, Rehovot 2002.

Pons, T.L., Welschen, R.A.M.: Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. – Plant Cell Environ. **25**: 1367-1372, 2002.

Raveh, E., Gersani, M., Nobel, P.S.: CO_2 uptake and fluorescence responses for a shade tolerant cactus *Hylocereus undatus* under current and doubled CO_2 concentrations. – Physiol. Plant. **93**: 505-511, 1995.

Repo, M.E., Huttunen, J.T., Naumov, A.V., Chichulin, A.V., Lapshina, E.D., Bleuten, W., Martikainen, P.J.: Release of CO_2 and CH_4 from small wetland lakes in western Siberia. – Tellus **59B**: 788-796, 2007.

Rodeghiero, M., Niinemets, U., Cescatti, A.: Major diffusion leaks of clamp-on leaf cuvettes still unaccounted: how erroneous are the estimates of Farquhar *et al*. model parameters? – Plant Cell Environ. **30**: 1006-1022, 2007.

Suh, S.U., Chun, Y.M., Chae, N.Y., Kim, J., Lim, J.H., Yokozawa, M., Lee, M.S., Lee, J.S.: A chamber system with automatic opening and closing for continuously measuring soil respiration based on an open-flow dynamic method. – Ecol. Res. **21**: 405-414, 2006.

Tissue, D.T., Nobel, P.S.: Carbon relations of flowering in a semelparous clonal desert perennial. – Ecology **71**: 273-281, 1990.

Wang, K.-Y., Kellomaki, S., Li, C.Y., Zha, T.S.: Light and water-use efficiencies of pine shoots exposed to elevated carbon dioxide and temperature. − Ann. Bot. **92**: 53-64, 2003.

Zha, T., Xing, Z., Wang, K-Y., Kellomaki, S., Barr, A.G.: Total and component carbon fluxes of a scots pine ecosystem from chamber measurements and eddy covariance. – Ann. Bot. **99**: 345-353, 2007.