Improvements in parameter estimates in the LI-COR LI-6800 portable photosynthesis system

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Introduction

Gas exchange offers a powerful, non-destructive tool for scientists to investigate plant photosynthesis. Like all techniques, care needs to be taken to reduce measurement errors and uncertainties that can occur in gas exchange measurements (Long et al. 2003, Pons et al. 2009, Flexas et al. 2007). Because of this, instrument manufacturers strive to improve instrument uncertainty when designing new instruments. In the case of gas exchange instrumentation, this uncertainty is generally ignored when logging discreet data points for survey or response curve measurements. Nevertheless, instrument uncertainty is propagated into both total measurement uncertainty as well as the uncertainty in calculated downstream parameters. Therefore, decreases in instrumental uncertainty are desirable to improve computed parameter estimates. Here, decreased instrument uncertainty in the LI-6800 is shown to translate into less uncertainty in derived parameters such as assimilation (A) when compared to the LI-6400XT. This reduced uncertainty also results in reduced uncertainty in the calculated quantum yield.

Materials and methods

To generate the light-response data, experiments were conducted on greenhouse grown *Nicotiana tabacum L*. 'Virginia Bright Leaf' and *Zea mays* (Genuity VT Triple Pro field corn). Plants were grown under well-watered conditions with a 15:9 h day/night cycle, and a relatively constant greenhouse temperature of 23°C. Gas exchange measurements used a LI-6400XT Portable Photosynthesis System (PSC-2928) and a LI-6800 Portable Photosynthesis System (68H-581003/68C-571003). The LI-6400XT was equipped with the 6400-04 fluorometer and the LI-6800 was equipped with the 6800-01 fluorometer. During measurements, instruments were arranged either directly horizontally opposed across the midrib on the same leaf or sequentially on the same leaf and as close together as possible in order to minimize the impact of biological variation. Light response data was gathered using the "Timed Lamp 2" auto-program in the LI-6400XT and the "Autolog – GenLoop" auto-program in the LI-6800. Full light response curves on tobacco used 13 different incident light levels (2000, 1500, 1000, 800, 600, 400, 200, 100, 80, 60, 40, 20, 0 µmole m^{-2} s^{-1}) while quantum yield experiments on corn used five incident light levels (100, 80, 60, 40, 20, and 0 µmole m^{-2} s^{-1}). In both instruments, leaf temperature was controlled at 26°C and sample CO_2 was controlled at 400 ppm. Relative humidity was manually kept at 50% or greater in the LI-6400XT during the full light response curves and 60-65% during the quantum yield experiments; in the LI-6800 automatic relative humidity control was set to 65% for all experiments.

Data was collected at 0.5 Hz for the full light response curves on tobacco and at 1 Hz for the quantum yield experiments on corn. The auto-programs were set to run for 180 s at a given light level before moving to the next light level in the auto-program. At each light level, the final 40 s of data was used as the representative steady-state data population that was subsequently analyzed. A program was written in Visual Basic for Applications to help sort and analyze the data in Microsoft Excel. Additional statistical data analysis was conducted using STATA/IC 15.

Results

Data from a typical light response curve from tobacco is shown below (Figures 1-3).

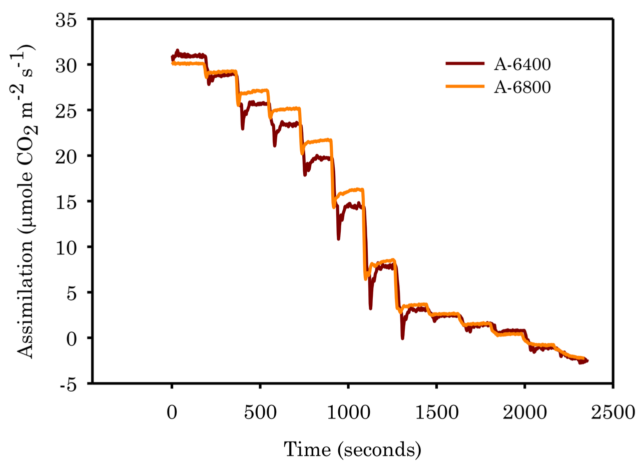

Figure 1. Typical CO_2 assimilation data vs. time from a light response curve conducted on tobacco.

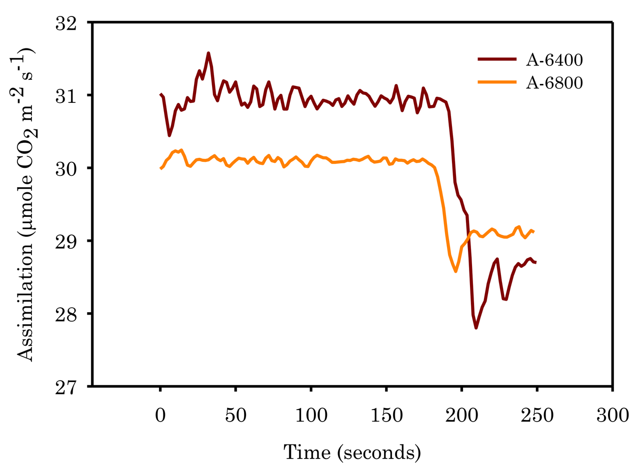

Figure 2. Enlarged portion showing the first 250 s of data from Figure 1. Differences in stability characteristics and system response to a change in light level are evident.

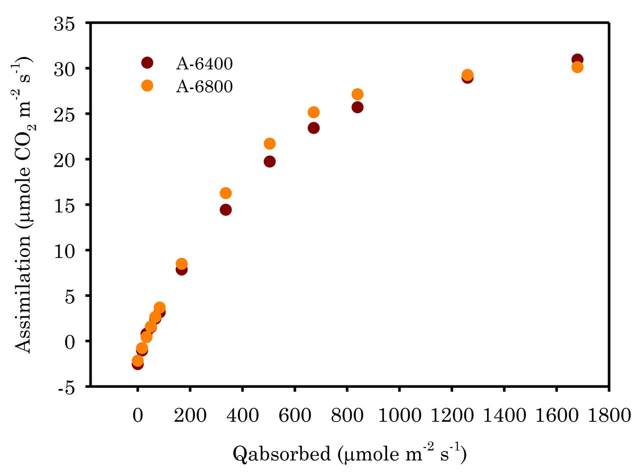

Figure 3. Overall A/Q data from a typical light response curve based on mean values from the steady-state data shows similar results from both instruments.

What drives differences in variability?

Assimilation is fundamentally derived from the following mass balance:

$$u_1c_1 - u_2c_2 - sA = \frac{dC}{dt} \qquad \text{Eq. 1}$$

When the above equation is solved for assimilation under steady-state conditions, the following results:

$$A = \frac{u_1(c_1 - c_2)}{s} + c_2E \qquad \text{Eq. 2}$$

What drives differences in variability? (continued)

Equation 2 represents a form of the equation that is used to calculate CO_2 assimilation in gas exchange instruments. To understand assimilation variability, it's useful to examine behavior of the individual input variables, which is shown in Figure 4 a-d below. The data shows differences between the two instruments and indicates that the reduced reference and sample CO_2 variability in Figure 4a and 4b is largely responsible for reduced assimilation measurement variability.

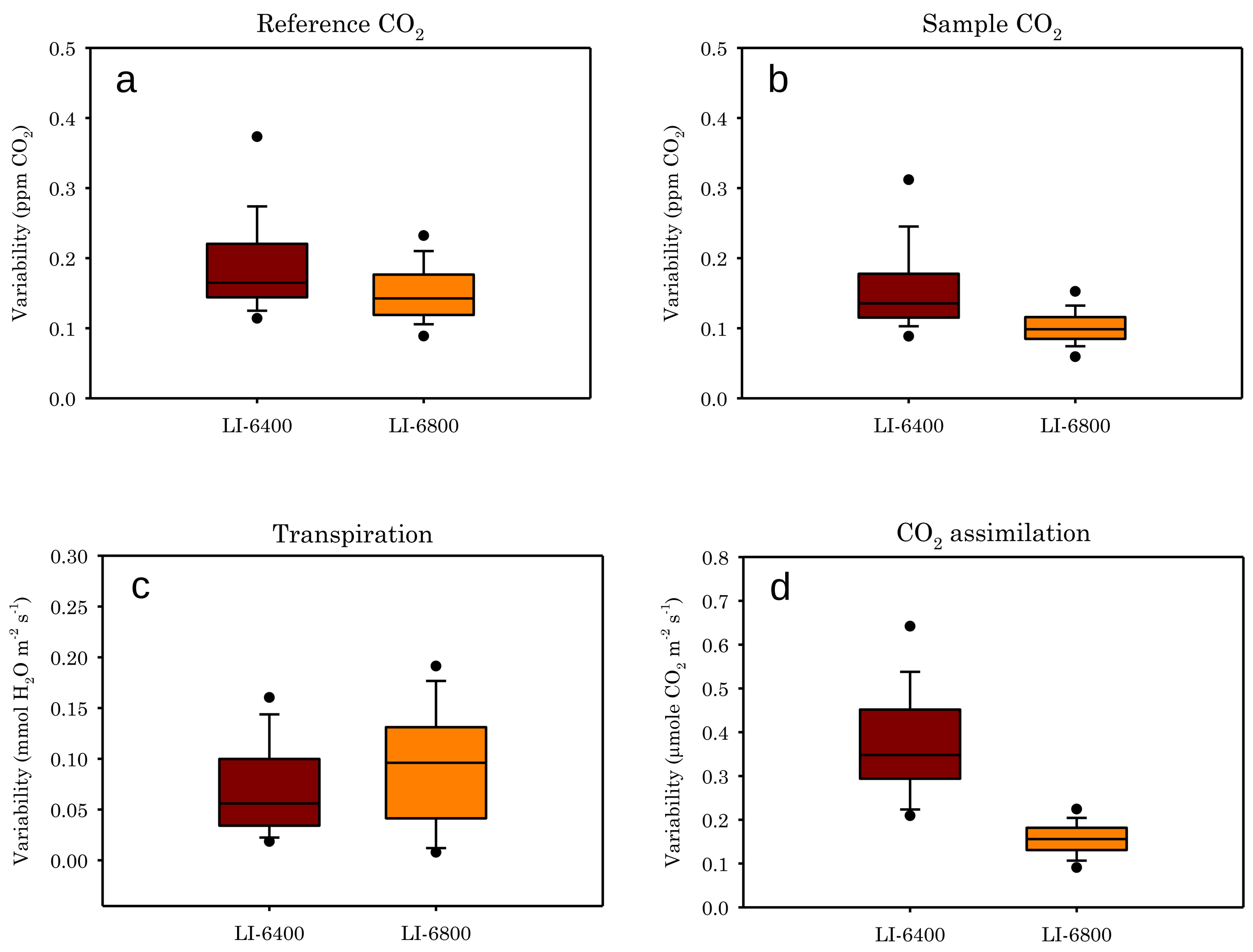

Figure 4 a-d (left) shows box plots of the peak-to-peak values of inputs to the assimilation equation from the 40 s steady-state data populations across all of the light curves conducted on tobacco. Figures 4a and 4b show that variation in reference and sample CO_2 is lower in the LI-6800 than in the LI-6400XT. Figure 4c shows transpiration variability was similar across both instruments. Overall assimilation values (Figure 4d) showed lower variability in the LI-6800.

Quantum yield determination in corn

Results from quantum yield experiments using corn are shown below:

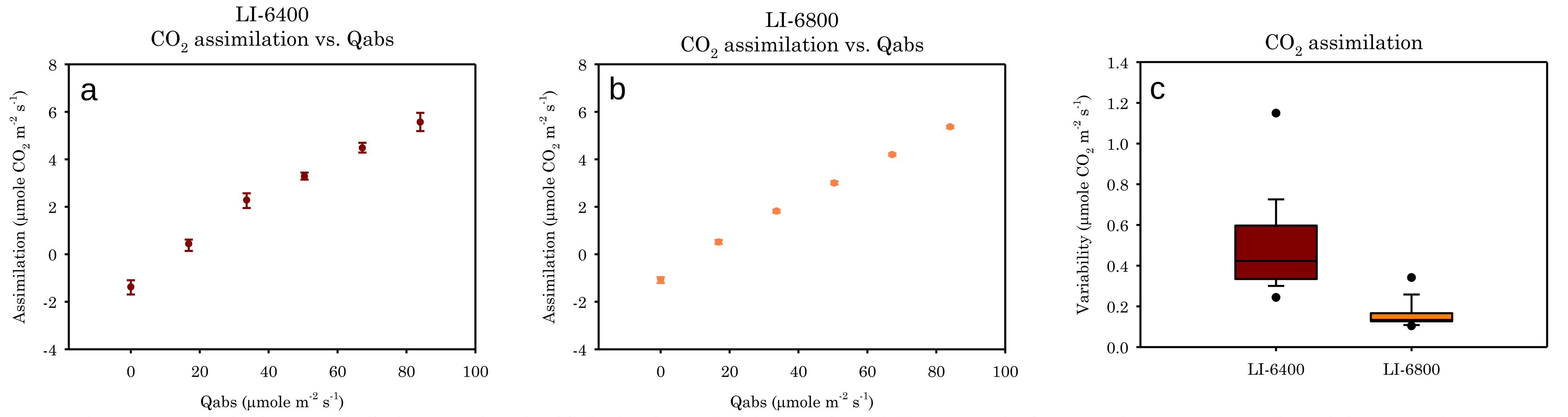

Figure 5a and 5b show assimilation vs. absorbed light for the LI-6400XT and LI-6800, respectively. Error bars represent the minimum and maximum values. Figure 5c shows a box plot of the peak-to-peak variability in the assimilation data for both instruments across all quantum yield experiments.

Figure 5a and 5b show data from a representative quantum yield experiment. The error bars show the peak-to-peak variability in the steady-state data for each data point. The impact that reduced variability in CO_2 assimilation (Figure 5c) has on the calculated quantum yield and 95% confidence intervals is shown in the following tables:

Table 1. LI-6400XT results

Trial	Φ (Mean)	r^2	95% CI (Lower)	95% CI (Upper)	Φ (High var.)	r^2	95% CI (Lower)	95% CI (Upper)
1	0.0838	0.976	0.0676	0.1	0.0821	0.957	0.0606	0.104
2	0.0836	0.977	0.0679	0.0994	0.0831	0.955	0.0609	0.105
3	0.0813	0.979	0.0667	0.0961	0.0777	0.953	0.0564	0.0989
4	0.0772	0.976	0.0622	0.0922	0.0751	0.962	0.0566	0.0936

Table 2. LI-6800 results

Trial	Φ (Mean)	r^2	95% CI (Lower)	95% CI (Upper)	% Reduct.	Φ (High var.)	r^2	95% CI (Lower)	95% CI (Upper)	% Reduct.
1	0.0759	0.998	0.0712	0.0807	70.7	0.0744	0.997	0.0695	0.0792	77.6
2	0.0752	0.996	0.0693	0.0811	62.5	0.0738	0.997	0.0689	0.0786	78.0
3	0.0756	0.996	0.0696	0.0816	59.2	0.0746	0.996	0.0690	0.0803	73.4
4	0.0768	0.996	0.0708	0.0827	60.3	0.0755	0.995	0.0688	0.0822	63.8

Table 1 and Table 2 show quantum yield determinations from the LI-6400 XT and LI-6800, respectively. The 95% confidence intervals (CI) are larger for the LI-6400 than for the LI-6800. In the high variability scenario, where data was chosen to deliberately maximize variability, the difference between the two instruments was most extreme. Overall, the LI-6800 data had 95% confidence intervals that were 59-78% smaller than those from the LI-6400XT data.

Conclusions

- **Control loop and IRGA improvements result in LI-6800 data having reduced instrument variability when compared to the LI-6400XT.**
- **Careful comparisons result in similar data from both instruments, but with different underlying measurement uncertainties.**
- **Quantum yield results were within the range of reported values (Hogewoning et al. 2012, Singsaas et al. 2001) and show that instrumental measurement uncertainties directly translate into different confidence limits for physiologically important parameters.**

References:
Flexas, J., A. Díaz-Espejo, J. Berry, J. Cifre, J. Galmés, R. Kaldenhoff, H. Medrano and M. Ribas-Carbó (2007). "Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization." *Journal of Experimental Botany* 58(6): 1533-1543.
Hogewoning, S. W., E. Wientjes, P. Douwstra, G. Trouwborst, W. van Ieperen, R. Croce and J. Harbinson (2012). "Photosynthetic Quantum Yield Dynamics: From Photosystems to Leaves." *The Plant Cell* 24(5): 1921-1935.
Long, S. P. and C. J. Bernacchi (2003). "Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error." *Journal of Experimental Botany* 54(392): 2393-2401.
Pons, T. L., J. Flexas, S. von Caemmerer, J. R. Evans, B. Genty, M. Ribas-Carbo and E. Brugnoli (2009). "Estimating mesophyll conductance to CO_2: methodology, potential errors, and recommendations." *Journal of Experimental Botany* 60(8): 2217-2234.
Singsaas, E. L., D. R. Ort and E. H. DeLucia (2001). "Variation in measured values of photosynthetic quantum yield in ecophysiological studies." *Oecologia* 128(1): 15-23.