PP SYSTEMS

Application Note #2010-02

Projected leaf area and flow rate - options and observed effects on gas exchange measurements with CIRAS-2

From a practical and theoretical standpoint, the objective in using gas exchange technology is to capture representative plant/soil physiological data, holding instrument error to a minimum. A basic goal in this is to ensure that sampling reflects real responses to natural or imposed environmental variability (treatments). Whether from an observational or experimental approach, the physiological response is not typically manipulated beyond the treatment, as this entails the risk of masking the potential treatment effect itself.

In relation to this, two gas exchange sampling parameters that receive frequent attention are i) leaf area of the cuvette and ii) the choice of flow rate during a given measurement session. This application note is intended to address some of the most common concerns related to these important parameters for which the researcher makes individual experimental design decisions. Both parameters figure prominently in the near real-time results generated by CIRAS-2's dedicated gas exchange equations (PP Systems 2010). Therefore, both are crucial factors when considering intercomparability of data when a likely or intended change of either leaf area (LA) or flow rate (*V*) occurs within an experiment.

The question arises: why switch between head plates during a given measurement session or experiment? Numerous circumstances can lead to the decision to change this sampling parameter. For example, a repeated measures design characterizing single-species physiology through different phases of leaf expansion might involve changing head plates over a relatively short period (weeks). Multi-species sampling of both narrow- and broad-leaved plants might require use of different head plates during one measurement session. An alternate approach would be to avoid use of different head plates altogether, opting instead to sample unknown leaf areas (LA not defined by the inside dimensions of the head plate), and determining LA post-measurement. This also requires selection of the Energy Balance option for leaf temperature determination – this is necessary because CIRAS-2's built-in infrared leaf temperature sensor is accurate only if the entire leaf chamber is filled, and no light is incident on the sensor itself.

Similarly, why not use a consistent flow rate throughout the experiment? Two circumstances come to mind: minimal *V* is advantageous when inducing or encountering very low photosynthetic rates, for example, associated with shade-tolerant species, or when sampling under marginally photosynthetic light intensities. Higher *V* can be used if chamber humidity is too high (>70% RH) to increase the volume flow through the chamber of the dry reference gas stream, but this is not normally needed when diverting the reference air through the desiccant columns (Envirogel). Additionally, chamber humidity settings can and sometimes should be changed to hold sampling conditions constant *inside* the leaf chamber, while ambient vapor pressure *outside* the leaf chamber fluctuates during measurements diurnally or daily.

Looking at the mass flow equation we see that LA (*a* term in the equation) has the immediate effect of decreasing calculated flow with increasing LA. Flow rate (V_{20} term in the equation) has the opposite relationship. Of the various combinations of LA and *V* we find that the highest possible mass flow per unit leaf area is achieved with LA=1.75 and *V*=470, yielding *W*=1.84 mol m^{-2} s^{-1}:

$$W(mol\ m^{-2}s^{-1}) = \left(\frac{V_{20}}{60 \times 10^3}\right) \times \left(\frac{1}{22.414}\right) \times \left(\frac{273.15}{293.15}\right) \times \left(\frac{1000}{1013.25}\right) \times \left(\frac{10^4}{a}\right)$$

These effects translate through the subsequent equations used to calculate transpiration, stomatal conductance, net photosynthesis and intercellular CO_2 concentration.

To avoid artificial results one should ideally maintain identical ratios of *V*:LA, if in fact changing either of these parameters becomes necessary. A simple rule and the easiest to remember is *V*:LA=10, where associated *W*=0.684 mol m^{-2} s^{-1}. This is most easily achieved by the following combinations of *V* with a given head plate size (Fig. 1). Since the operational range of *V*=150-470 ml min^{-1}, there is some room for variable settings while maintaining equivalent ratios.

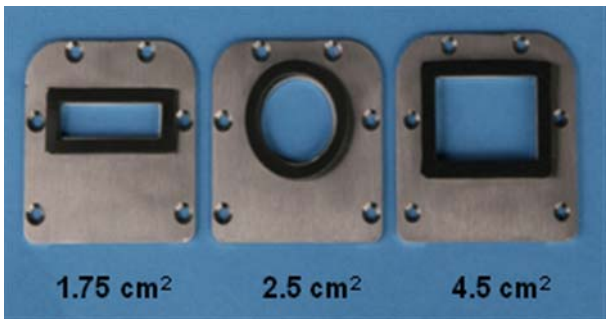

Figure 1.

175 ml min^{-1}:1.75 cm^2	Small rectangular headplate (25 x 7 mm)
250 ml min^{-1}:2.5 cm^2	Broadleaf circular headplate (18 mm dia)
450 ml min^{-1}:4.5 cm^2	Large rectangular headplate (25 x 18 mm)

In order to illustrate the difficulties of intercomparability, data was recorded using each of the three head plates (without maintaining the same *V*:LA ratio) (Fig.2). In this example *V* was held constant at 300 ml min^{-1} as was the entered value of LA=1.75 cm^2, while physically altering the true leaf area sampled with different head plates. Net photosynthesis was recorded for each leaf area assay within each of four LED light levels after intial stabilization of the leaf at a saturating light intensity and 25 °C. The four light levels were applied and decreased sequentially to ¼ of the previous intensity, PPFD indicated by the subscript of Q. All measurements were taken at the same leaf position from a single leaf of *Nicotiana tabacum* cv. Bel-B.

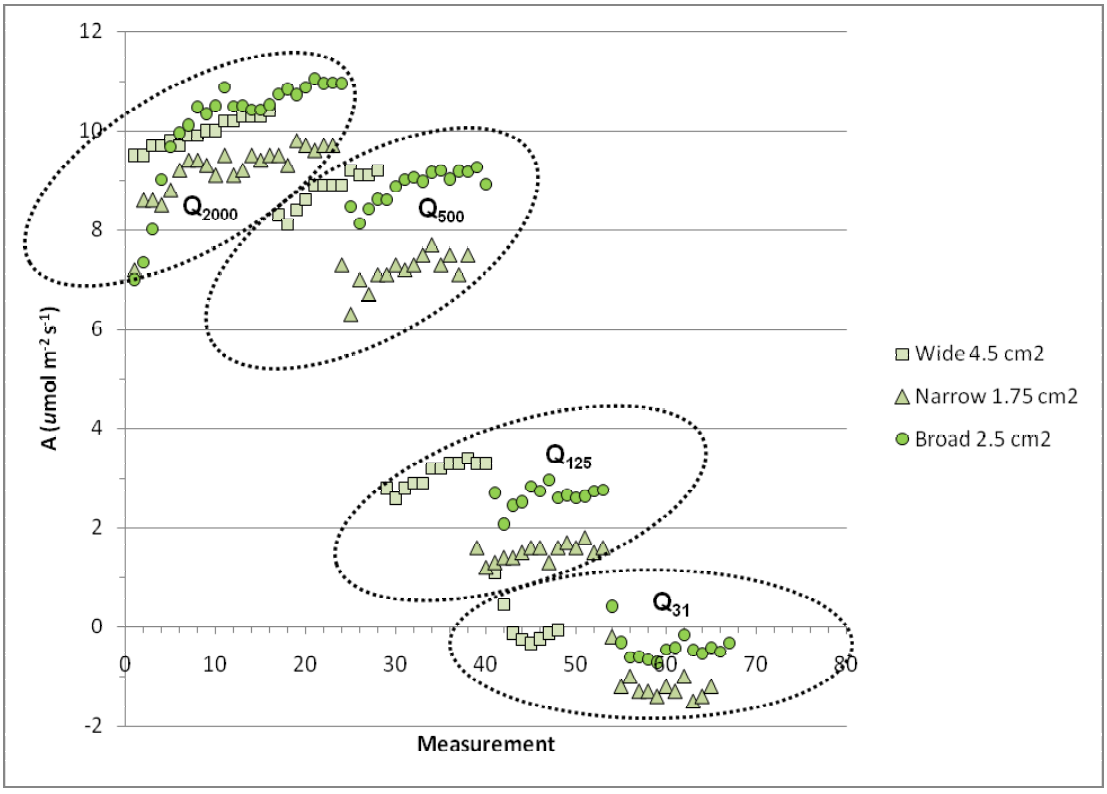

Figure 2.

The resulting mean differences in calculated net photosynthesis are as large as 42.8% (data not shown). A second example (Fig. 3) illustrates how inappropriately scaled *V*:LA affects calculated stomatal conductance (g_s). In the extreme case of LA=4.5 cm^2 data calculated based on LA=1.75 cm^2, the uncorrected data (diamonds) are far outside the normal range of values. When recalculated based on the

correct LA (circles), data are comparable to those recorded with the initial correct settings in CIRAS-2 parameters (squares).

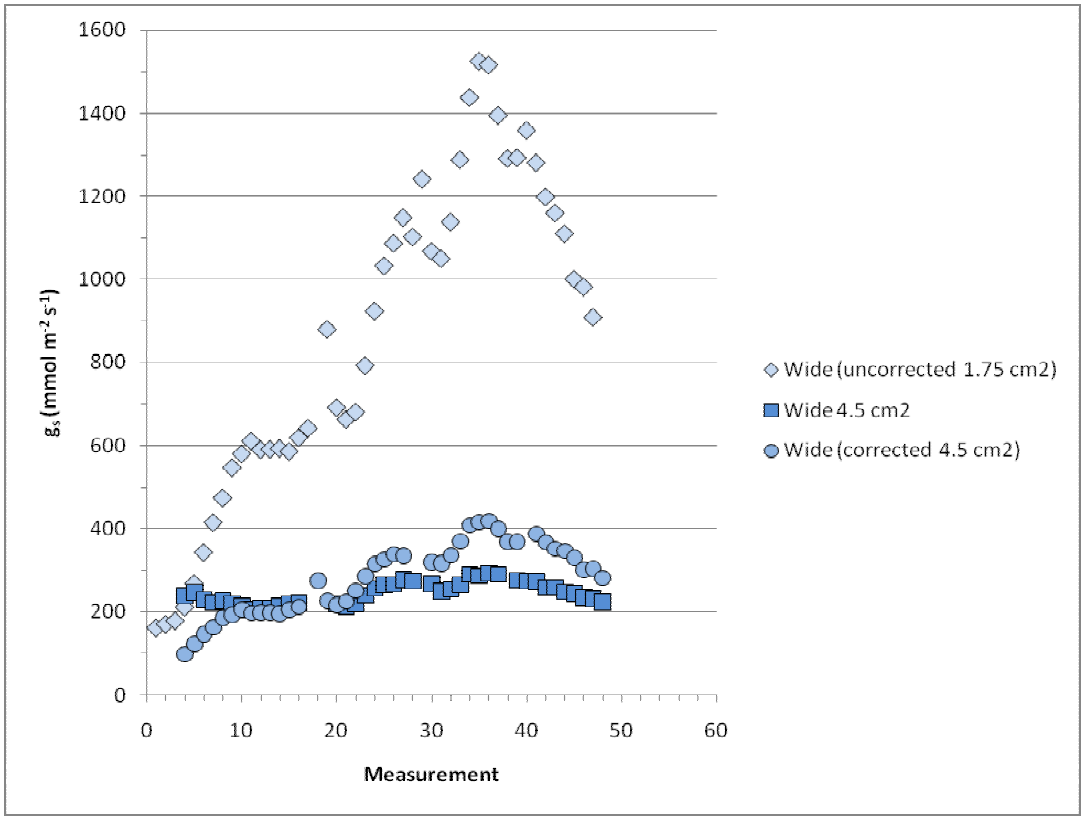

Figure 3.

In contrast, good reliability across leaf area sampled is illustrated by Figure 4. The figure represents typical sample data where *V*:LA=10 was applied as shown above using the three head plates. Data were recorded in the field under two qualitative ambient light conditions with approx. PPFD of 1250-1550 (Sun) and 200-300 (Shade) µmol m^{-2} s^{-1}. All measurements were taken at the same leaf position from a single leaf of *Helianthus annuus*. Shown are mean net photosynthetic rates ± one SD (n=9-12). No statistically significant differences were detected.

In addition to the factors already discussed, CO_2 control mode itself is crucial whenever it is likely or intended that *V* will not be held constant throughout an experiment. Using the 'Supply (Ref) Approximate ppm' control type, any change in *V* is accompanied by a change of proportional magnitude and direction in the reference CO_2 concentration (Cr), requiring an appropriate adjustment of the Cr ppm setpoint value. Because of this it is often desirable to use the *Supply (Ref) Set ppm* CO_2 control type, which requires only a short Cr readjustment phase following each adjustment of flow rate.

Of the three head plate (LA) options, a good reason for selecting the 4.5 cm^2 head plate whenever possible is that it has the practical advantage of providing both the largest leaf area sampled and highest leaf area:gasket area ratio. This can be important when precise measurements are required under low physiological rates such as dark respiration, when minute diffusion of CO_2 beneath and through gaskets may confound the calculated rates (Pons and Welschen 2002, PP Systems Application Note 2010-03).

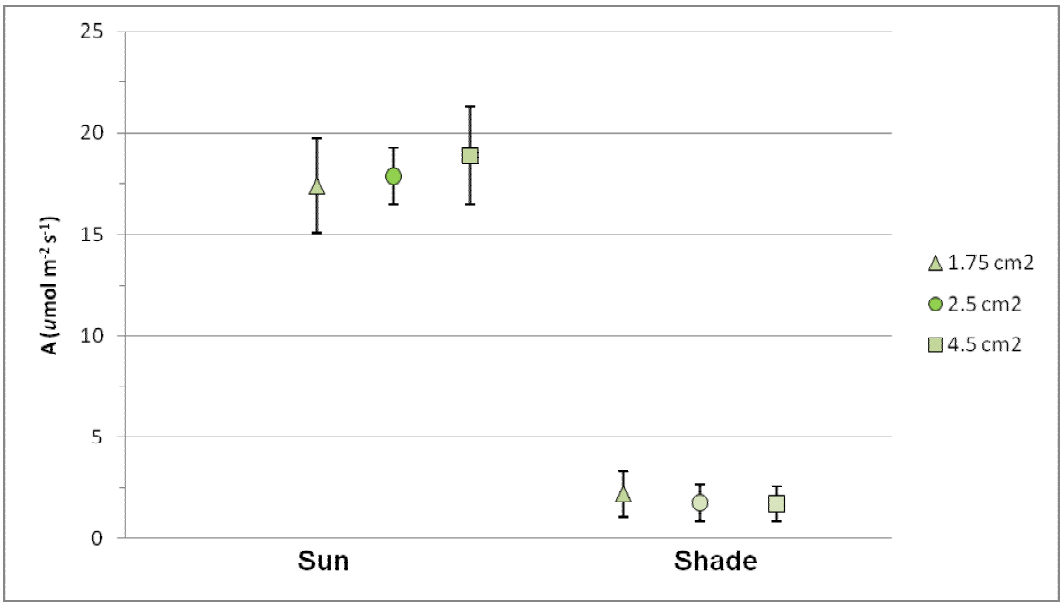

Figure 4.

Acknowledgements:

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References:

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

PP Systems Inc. 2010. CIRAS Gas Exchange Equations (For CIRAS-1 and CIRAS-2). Version 3.0.

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