Using the 6400-17 Whole Plant Arabidopsis Chamber

APPLICATION NOTE

Summary

- The Whole Plant Arabidopsis (WPA) Chamber measures whole plant CO_2 exchange.
  - Rosette photosynthesis can be isolated using a clay cap and a slight overpressurizing of the chamber.
- Effective for healthy Arabidopsis plants larger than 1.5 cm^2 total leaf area.
- Only rosette-level parameters should be examined (e.g. rosette transpiration instead of leaf transpiration).
- Environmental conditions in the WPA Chamber should be chosen in relation to growth conditions.

Arabidopsis and Photosynthesis

Advances in molecular and genomic techniques in plant biology have provided researchers with diverse tools to study metabolic and photosynthetic processes in plants. The genome of *Arabidopsis thaliana* is among the most widely studied of all plant species. With the wealth of genetic information and tools available, studies of the genetic basis of physiological pathways can now be undertaken with greater ease. The new 6400-17 Whole Plant Arabidopsis (WPA) Chamber provides scientists with a novel tool for exploring photosynthetic capacity, respiration rates and other gas exchange parameters among mutant collections of *A. thaliana* and other short-stature species.

Gas exchange studies of small rosette plants have been limited because leaves with short petioles are difficult to enclose within a clamp-style chamber and because small leaf areas lead to large uncertainties in gas exchange measurements. The WPA Chamber circumvents these issues by enclosing the entire rosette within the chamber, thereby alleviating the need for narrow approach angles and increasing the total leaf area that can be assessed. However, by enclosing the whole plant and container, net CO_2 flux is the combination of rosette photosynthesis, plant respiration, plant growth medium flux and container sorption. In some studies, this approach is exactly what is desired, such as assessments of net plant carbon exchange (net CO_2 uptake = photosynthetic uptake – all respiratory losses). If researchers are interested in photosynthetic capacity alone, then the rosette must be isolated.

When examining isolated rosette gas exchange, care should be taken to differentiate between canopy level and leaf level processes. To illustrate, stomatal conductance (g_s) is not calculated by the LI-6400XT when the WPA Chamber is installed because the rosette structure makes an assessment of leaf boundary layer conductance nearly impossible. The low-lying leaves of a rosette slow air movement around each leaf, thereby increasing the boundary layer (the calm air layer around each leaf). The rosette also slows air movement over the plant, creating a second layer of still air over the entire plant. To calculate g_s, the boundary layer conductance is assumed to be very high (i.e. not limiting), allowing it to be considered negligible in the calculation of total resistance to evapotranspiration (see LI-6400XT Manual, Chapter 1 for a complete description). However, since the total water lost by the plant is measured, an assessment of canopy transpiration can be made. In most instances, canopy level parameters are measured with the WPA Chamber, rather than leaf level parameters.

Plant culture practices – Separating rosette and planting medium flux

In many research settings, Arabidopsis is cultured in small containers filled with a peat-based planting medium that is kept at or near water saturation. These plants are

grown indoors in greenhouses or in growth cabinets with low light levels (many growth cabinets are < 200 μmol m^{-2} s^{-1}, approximately 10% full sunlight). The combination of these conditions can lead to bacterial and/or algal growth in the planting medium. While not necessarily detrimental to the plant, this does have an impact on CO_2 flux. Therefore, it is important to have a method of isolating the CO_2 flux from the planting growth medium.

We have developed several methods to suppress CO_2 flux from the plant growth medium for the 6400-17 WPA Chamber. These methods can be used individually to block much of the CO_2 flux, or in combination to ensure that there is no flux from the planting medium.

1. Plant Culture: Arabidopsis must be grown in either 4 cm diameter Cone-tainers™ or 6.5 cm diameter pots for use in the WPA Chamber. Once the planting medium is in the container, the surface of the medium can be sealed by several gas-tight methods. The preferred method is to use standard pottery clay to create a 4 - 6 mm thick layer on top of the medium that adheres to the containers' sidewalls. A 5 - 8 mm hole is made in the center of the clay disk through which either seeds can be planted or 5 - 10 day old seedlings can be transplanted from agar plates or planting medium (Figure 1A). As long as the planting medium remains moist, the clay will also and will provide a good germination/growth surface for the young plants. As the plant grows, it will occlude the hole, suppressing any CO_2 flux through the planting medium.

A slightly messier alternative is to spread petroleum jelly across the surface of the planting medium. This is also quite effective at blocking CO_2 influx from the medium, but is more difficult to work with in general. From testing, we found that plastic film was not a suitable alternative because plants tended to germinate underneath the film. Additionally, plastic film did not seal around the plant as well since the hole size has to be cut to accommodate mature plants. This hole acted as a venturi and drew CO_2 from the plant medium at higher rates than just the medium alone.

A:

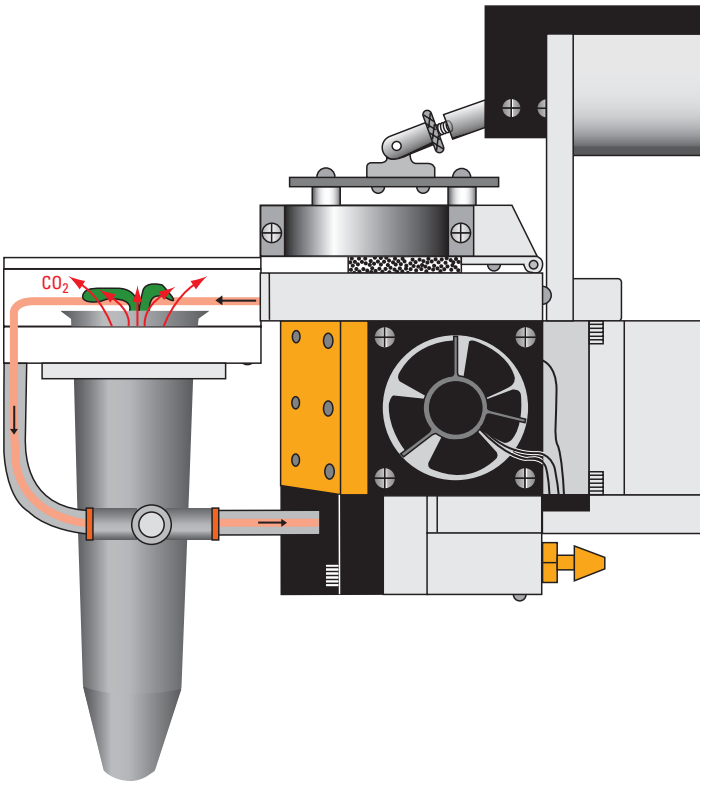
B:

Figure 1: *A typical Arabidopsis plant measured in the WPA Chamber has a rosette diameter of 5 – 7 cm. The clay cap on top of the planting medium prevents CO_2 from diffusing into the chamber during measurements.* ***A:*** *7-day post-germination Arabidopsis seedling transferred into hole in the clay cap.* ***B:*** *Arabidopsis after 4 – 5 weeks of growth in container. Total leaf area is 10 cm^2, which is large enough to provide a good ΔCO_2 for measuring photosynthesis with limited self-shading of the leaves.*

2. Exhaust Flow Restrictions: The LI-6400XT is an open, flow-through system where environmentally conditioned air enters the chamber, forcing an equal volume out the exhaust path. The WPA Chamber incorporates a flow-restricting needle valve (Adjustable Exhaust Tube Assembly, LI-COR part #9964-118) into this path that allows the user to adjust the flow through the exhaust (Figure 2). By restricting the exhaust flow, air is forced through the plant medium, which suppresses CO_2 flux from the medium. Typically, closing the needle valve to 25 to 50% is sufficient to divert 100 – 150 μmol s^{-1} of flow through the medium, while maintaining enough flow through the match valve to match the IRGAs.

Forcing air to flow through the medium can increase pressure within the chamber due to resistance to flow through the plant growth medium. To prevent excessive overpressure within the WPA chamber, the user should perforate the container with one or two 0.3 mm holes located 1.5 – 2 cm below the top of the container (Figure 2B). Standard push-pins for bulletin boards work well for this purpose. These holes function as exhaust paths and reduce the backpressure on the system because of the decreased pathlength through the plant medium. Matching the IRGAs will remove any small offset in CO_2 and H_2O measurements due to the slight pressure increase.

A:



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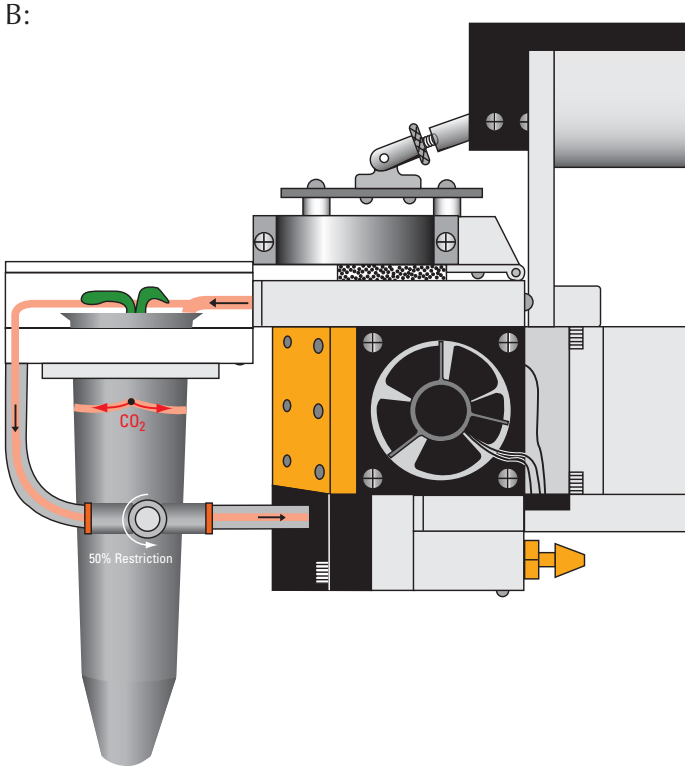

Figure 2: *Exhaust path of the WPA Chamber with the optional Adjustable Exhaust Tube Assembly. The needle valve regulates flow through the exhaust tube, suppressing CO_2 flux from the medium.* ***A.*** *An unrestricted (Open) exhaust path valve allows all air flow to exhaust the system via the normal pathway. CO_2 fluxes freely into the chamber from the roots, plant medium and outside the container.* ***B.*** *The addition of a small vent hole and restriction of the needle valve to 25 – 50 % decreases influx. Some of the flow is diverted through the plant medium and out the vent, thereby suppressing flux into the chamber.*

For plants that are not needed for seed production, additional tests or assays, the CO_2 flux contributions from the plant growth medium and/or roots can be measured directly. To measure this CO_2 flux, the Arabidopsis plant should be measured in the chamber without making any of the adjustments above (use the Unrestricted WPA Chamber Exhaust Tube, LI-COR part# 6564-287). Once all measurements of the plants photosynthetic/respiration rate are completed, the vegetative portion of the plant is removed and the remaining flux can be measured. This flux from the plant medium and roots is then subtracted from the total measured with the intact plant.

Gas exchange nuances – Getting the best measurements

The CO_2 concentration in growth cabinets and greenhouses can be higher than current ambient conditions (385 μmol mol^{-1} in 2008), commonly ranging from

600 – 800 μmol mol^{-1} or more. Plants grown in elevated CO_2 adapt by decreasing stomatal density and aperture, decreasing Rubisco content, increasing starch storage, and other physiological adaptations (Curtis and Wang, 1998; Medlyn et al., 1999; Ainsworth et al., 2002). Therefore, it is important to configure the LI-6400XT CO_2 mixer to replicate the growth conditions to get the best measurement of plant CO_2 assimilation and/or respiration.

To normalize values for differences in leaf size and number, most gas exchange parameters are reported per leaf area. For Arabidopsis rosettes, either photographic analysis or destructive leaf area measurements can be used. Precise, destructive measurements of Arabidopsis leaf area can be made using a leaf area meter such as the LI-3100C, which has a resolution of 0.1 mm^2 (http://www.licor.com/env/Products/AreaMeters/LI-3100C/3100C_intro.jsp). Non-destructive measurements of leaf area can be conducted with the photographic method using software such as "ImageJ," offered for free by the National Institute of Health (http://rsbweb.nih.gov/ij/). A uniform, white background behind the rosette increases the contrast for image analysis.

Experimental Validation – Protocol and Results

Validation of the WPA Chamber was made with both 4 cm Cone-tainers™ and 6.5 cm pots. Wild-type Arabidopsis (Col-0) was used in all tests involving plants. The plants were germinated on Miracle-Gro Potting Mix™ (forest compost, peat moss, perlite, wetting agent and 0.21-0.07-0.14 N-P-K) and transplanted 5 – 10 days post germination. The plants were grown under 175 μmol m^{-2} s^{-1} fluorescent light for 8-hour short days to prevent bolting. For most of the following experiments, 6 to 8 week old plants (5 – 7cm rosette diameter, Figure 1B) were used.

Mass flow out of the WPA Chamber through the medium was assessed with a precision variable area flow meter (FL-3803ST-NV, Omega Engineering Inc., Stanford, CT) placed in the chamber flow line. The flow meter was calibrated against a LI-6400XT mass flow sensor and pressure changes due to restrictions were measured by differential pressure sensor (DGM-240169600, American Gage, Atlanta, GA). Air flow through the plant medium was calculated as the difference between inflow measured by the LI-6400XT mass flow meter and the outflow measured by the precision flow meter. For water-saturated peat (a common growth medium and also worst case scenario), there was essentially zero flow through the plant growth medium (Figure 3, closed triangle).

When a needle valve in the exhaust path was closed to 50%, the flow through the medium remained 0 μmol s^{-1} (Figure 3, closed square). However, when a small vent hole (~0.5 mm) was punched in the sidewall of the container about 2 cm below the top, the flow through the medium was 90 – 100 μmol s^{-1} (Figure 3, open triangles). Closing the needle valve to 50%, further increased the flow through the medium to 100 - 150 μmol s^{-1} (Figure 3, open squares). The amount of flow through the small vent hole will be dependent upon the plant growth medium type and the moisture content of the medium.

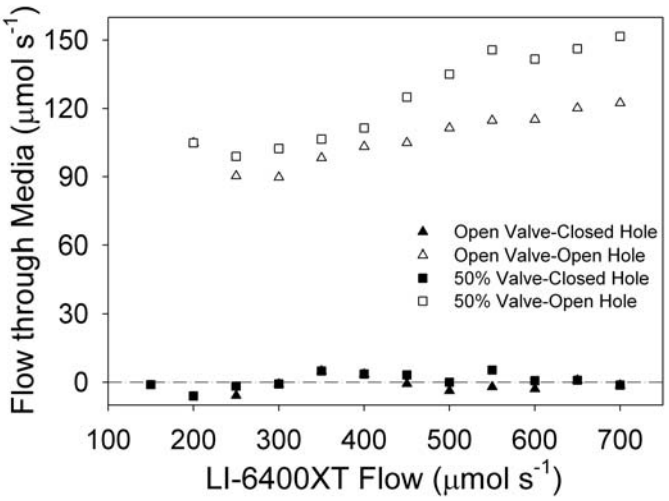

Figure 3: *The calculated flow through the plant growth medium. The flow through the medium is the difference between the expected flow rate (measured on the LI-6400XT) and the flow through the exhaust assembly. Tests were done with a water-saturated, peat medium. The exhaust restriction valve was either completely open or closed to 50%. The small vent holes were either plugged with plasticine (Closed Hole) or unplugged (Open Hole).*

Plant root and microbial respiration plus diffusion though the plant growth medium causes an apparent CO_2 flux into the WPA Chamber. Suppression of this flux is necessary to measure canopy-only CO_2 flux. Different capping methods were explored including plastic film and heavy pottery clay. Containers of plant medium were incubated for 3 weeks alongside growing plants. The medium was then tested for effective barriers to CO_2 flux into the chamber. Uncapped medium resulted in a 5.1 μmol mol^{-1} CO_2 increase in the chamber (Table 1). The influx of CO_2 increased 5-fold when the container was placed into water to prevent diffusion through the medium out the bottom and was comparable to completely H_2O-saturated medium. Covering the planting medium with plastic film (not shown) and a 4 mm-thick clay plug both suppressed CO_2 flux into the WPA Chamber. When a

hole was punched in either barrier to allow a plant to grow through, there was an increase in the CO_2 flux into the chamber. However, later tests showed the growing plant more effectively filled the hole in the clay than the hole in the plastic film. Additionally, the plastic film was more difficult to secure to the container and was often torn during insertion into and removal from the WPA Chamber.

Table 1: *Measured ΔCO_2 (CO_2Sample – CO_2Reference) for different methods of covering the plant growth medium. The bottom of the growth containers were placed in water to prevent any CO_2 flux from outside into or out of the chamber through the plant growth medium. The cap of heavy clay effectively blocked CO_2 flux from the plant medium.*

Media Covering	H_2O Corrected ΔCO_2
Uncovered	5.1 ± 0.89
In water	23.6 ± 0.07
Saturated growth medium	24.9 ± 0.13
In water with plastic film and 0.9 cm hole	13.3 ± 0.32
In water with heavy clay cap	1.3 ± 0.32
In water with clay cap and 0.9 cm hole	10.6 ± 2.65

Suppression of CO_2 flux from the plant growth medium was tested on media without plants and on media with 8-week old plants. The CO_2 concentration inside the chamber was matched to the room CO_2 concentration to remove any diffusion effects. Injections of 250 µl of pure CO_2 were made 0.5 – 2 cm below the plant medium surface through small vent holes in containers. Vent holes > 1.5 cm below the top of the container were not as effective at suppression of the CO_2 spike ($\Delta_{spike}CO_2$, data not shown). A second series of small vent holes were tested to assess aperture effects on the $\Delta_{spike}CO_2$ suppression. With greater plugging of the small vent hole, there were larger $\Delta_{spike}CO_2$ (Table 2). For the least occluded vent hole (Restriction Level 1), the $\Delta_{spike}CO_2$ was similar to the negligible CO_2 increase (0.1 µmol mol^{-1}) when CO_2-free air was injected into the plant growth medium. This is well within the specified IRGA peak-to-peak noise of 0.3 µmol mol^{-1} at 350 µmol mol^{-1}.

Table 2: *Test of pure CO_2 injection detection within the sample cell at different vent hole restrictions for 4 cm Cone-tainers™. The change in CO_2 following a 250 ml injection of pure CO_2 was measured as the $\Delta_{spike}CO_2$ (maximum CO_2 during spike – average CO_2 prior to injection). The holes in the side of the container (approx. 0.3 mm placed 1 to 1.5 cm from top of container) were either sealed with plasticine (Closed) or unsealed (Open). In the Open configuration, the needle was either not removed from the injection hole (Restriction Level 4), removed 1 – 2 seconds (Restriction Level 3) or immediately (Restriction Level 2) after injection, or the injection was made through a second, sealed hole thereby leaving the test hole completely unblocked (Restriction Level 1). Additionally, a mass flow increase caused by the injections was insufficient to cause the $\Delta_{spike}CO_2$ based on ambient air (not shown) and CO_2-free air injections. The clay cap consisted of a 3 – 5 mm thick clay cap with a 4 mm hole in the center. The values are the average $\Delta_{spike}CO_2$ ± St. Err. of the measure for the number of subsamples indicated (n).*

Injected gas	CLOSED Restriction Level 5	n	OPEN Restriction Level 4	n	Restriction Level 3	n	Restriction Level 2	n	Restriction Level 1	n
Pure CO_2	195.3 ± 21.8	5	138.4 ± 2.4	2	60.9 ± 22.1	3	9.4 ± 0.6	2	0.14 ± 0.06	5
CO_2-free air	-0.07 ± 0.29	2							0.11 ± 0.05	2
Pure CO_2 under clay cap	1.02 ± 0.36	3							0.15 ± 0.003	2

The effect of the clay cap and flow through the plant growth medium is apparent in the trace of CO_2 with the different techniques applied sequentially (Figure 4). Without flow through the plant growth medium and/or clay cap on the medium, injection of pure CO_2 resulted in a $\Delta_{spike}CO_2$ of 175 µmol mol^{-1} (Figure 4, Event 2). The combination of a clay cap with 0.9 cm hole for the plant on the plant medium and/or forcing part of the exhaust flow through the medium completely suppressed CO_2 flux into the WPA Chamber (Figure 4, Events 4, 6 and 8).

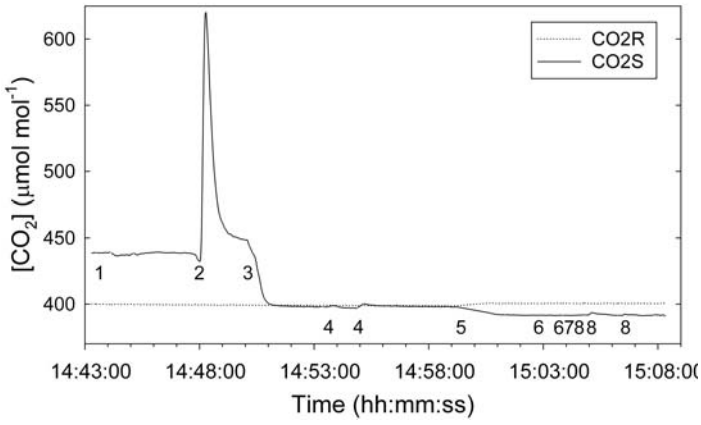

Figure 4: *Clay was tested as a barrier to CO_2 flux from the medium. Time trace of CO_2 concentrations for sample and reference IRGAs with the small vent hole plugged and unplugged for 4 cm Cone-tainer™. The exhaust needle valve was partially closed to increase the flow through the medium. This test used a second hole that was always plugged to inject CO_2. Description of events:*

1. *All holes plugged and needle valve 25% restricted.*
2. *Inject 250µl pure CO_2 (1x).*
3. *Small vent hole in container opened.*
4. *Inject 250µl pure CO_2 (2x).*
5. *Paused program to place clay cap on surface of media.*
6. *Inject 250µl pure CO_2 (2x).*
7. *Small vent holes in container closed.*
8. *Inject 250µl pure CO_2 (3x).*

After 8 weeks of growth in short days, Arabidopsis plants completely occlude the hole in the clay cap. The plant rosettes were also large enough to cover the uncapped containers' medium as well. The same pure-CO_2 injection technique was used to test whether the larger rosette acted as a barrier to CO_2 flux into the chamber. Plants that were grown in containers without the clay cap had the characteristic $\Delta_{spike}CO_2$ following an injection of CO_2 into the plant growth medium (Table 3). This spike was partially abated by restricting exhaust flow with the needle valve as seen in the previous experiments. When the rosette was removed, there was a slight increase in the $\Delta_{spike}CO_2$ suggesting that the rosette might suppress some of the CO_2 flux, but the effect is small and large rosette size is not an effective way to reduce the influx of CO_2 from the plant growth medium. The effectiveness of the clay cap was not improved by the large rosette covering the clay cap (Table 4). As Arabidopsis plants grew, they occluded the hole in the clay cap, further restricting the CO_2 flux from the plant growth medium.

Table 3: *Test of CO_2 injection detection within the sample cell for 4 cm Cone-tainers™. The flux is measured as the $\Delta_{spike}CO_2$. The tests were made with the needle valve either open or 50% closed. Plants were grown either with or without the clay cap in place. Measurements were made with the vegetative portion of the plant and again with the vegetative portion removed. Pressure in the chamber was either normal (about 20 Pa) or increased by restriction of the needle valve (overpressure; approx. 150 Pa). The values are the average $\Delta_{spike}CO_2$ ± St. Err. of the measurement for the number of subsamples indicated (n).*

	Rosette, Open	n	50% Closed	n	No Rosette, Open	n	50% Closed	n
Uncovered	144.0 ± 61.1	2	19.8 ± 14.2	2	192.2 ± 1.0	2	52.7 ± 13.3	2
Clay cap	0.16 ± 61.1	5	0.53 ± 0.3	6	0.33 ± 0.2	5	0.23 ± 0.2	5

With the complete suppression of $\Delta_{spike}CO_2$ by clay capping and flow restriction, we tested the smallest measurable plant size. Arabidopsis plants of different sizes were measured over one week. Total plant leaf area was measured by photographing the plant and analyzing the image with ImageJ software (NIH, http://rsbweb.nih.gov/ij/). The smallest plant had 0.7 cm^2 of leaf area (Figure 5B) and the largest had just over 10 cm^2 (Figure 1B). Light response curves were measured for all plants (Figure 6). The three larger-sized plants (1.7, 4.2 and 10.2 cm^2) produced consistent photosynthetic responses to increasing light. Photosynthetic rates of 2 - 4 µmol CO_2 mol^{-1} air at 100 to 200 µmol m^{-2} s^{-1} white light are comparable to previously reported rates (reviewed by Lake, 2004). The light compensation points for the entire rosette were 25 – 50 µmol m^{-2} s^{-1}. Dark respiration rates are within reported ranges of 1 to 2 µmol m^{-2} s^{-1} (Byrd et. al., 1992). However, the smallest plant (0.7 cm^2) was too small to produce consistent results. The ΔCO_2 (0.6 µmol mol^{-1} between reference and sample IRGAs) that was generated by these small plants was only slightly larger than the IRGA precision, meaning that up to 50% of the measurement could be IRGA noise. (See LI-6400XT Manual, Chapter 1 for formula.) Larger rosettes take up more CO_2 and thereby generate a larger ΔCO_2 (2.5 µmol mol^{-1} for the 1.7 cm^2 plant) which lessens the impact of IRGA precision on the measurement. Based on our results, rosettes of at least 1.5 cm^2 are large enough to make light saturated measurements of photosynthesis. However, other factors can impact photosynthetic rates such as mutation, abiotic stress and growth conditions, and may necessitate greater leaf area of larger plants.

A.

B.

Figure 5: *Arabidopsis in 4 cm Cone-tainers™ with clay caps over medium to block CO_2 flux from plant medium and roots. Plants are 3 weeks old.* ***A.*** *Total leaf area is approximately 1.5 cm^2, which is the minimum recommended size for measurements.* ***B.*** *Total leaf area is approximately 0.7 cm^2, which is too small to give adequate results.*

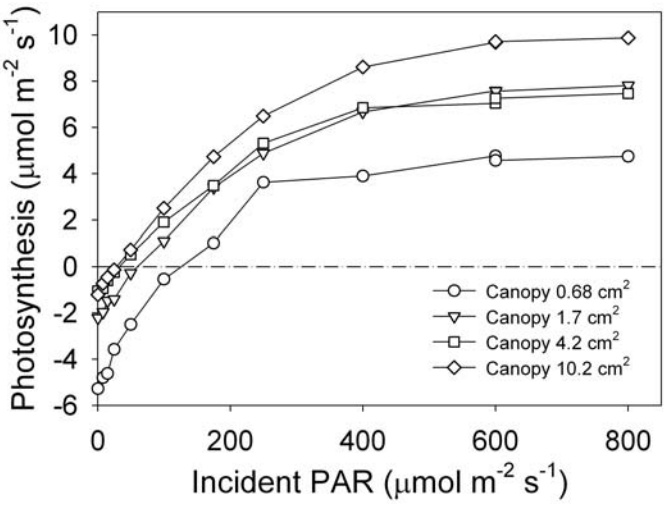

Figure 6: *Light response curves for Arabidopsis plants of different sizes. The responses were measured at constant water mole fraction and growth CO_2 concentration (600 μmol mol^{-1}).*

Conclusions

The WPA Chamber allows users to make rapid gas exchange measurements of either a whole plant or aboveground portion of *Arabidopsis thaliana*. Through a combination of cultural practices and instrument adjustment, CO_2 flux from the plant growth medium and roots can be suppressed, allowing for direct measurement of the rosette gas exchange. Since the entire rosette is enclosed in the chamber, individual leaves are not measured and therefore care should be taken not to use specific leaf-level parameters, such as stomatal conductance or intercellular CO_2 concentration. With careful selection and set-up of the environmental conditions in the chamber, accurate measurements of the Arabidopsis rosette can be made.

IMPORTANT NOTE: Although other sources for the following parts do exist, we recommend that you purchase directly from LI-COR, as many Cone-tainers and pots from other suppliers have similar specifications, but often do not fit our chambers.

LI-COR p/n for the 1.5" diameter Cone-tainer: 610-09645
LI-COR p/n for the 2.5" diameter pot: 610-09646

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