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Protocol for leaf-level gas exchange measurement for stomatal conductance model calibration V.2

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We use this protocol and it's working

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Abstract

Calibration of parameters in leaf-level stomatal conductance models is most commonly performed based on either leaf-level measurement of stomatal conductance from gas exchange instrumentation, or by inferring average stomatal conductance from whole-plant or -canopy scale measurement of transpiration and an associated effective vapor pressure deficit (VPD). When the stomatal conductance model is to be applied within a spatially explicit model such as a multilayer model (e.g., Ding et al., 2014) or three-dimensional plant model (e.g., Kim et al., 2020), the latter approach is generally preferred because it removes the impacts of canopy structure from the stomatal conductance parameters, which are presumed to reflect the intrinsic stomatal physiology.

Instrumentation for measuring leaf-level stomatal conductance is well-developed and has been widely used for decades. However, high-quality data sets enabling consistent model parameter sets across species remain sparse. As stomatal conductance is a critical input to crop, land surface, and Earth system models, there is a growing need to collect reliable, representative data (M'arquez et al., 2025) and to systematically catalog model parameters across species (Miner and Bauerle, 2017). A shared protocol of efficient collection of quality data for stomatal model parameter estimation could help the research community meet this demand.

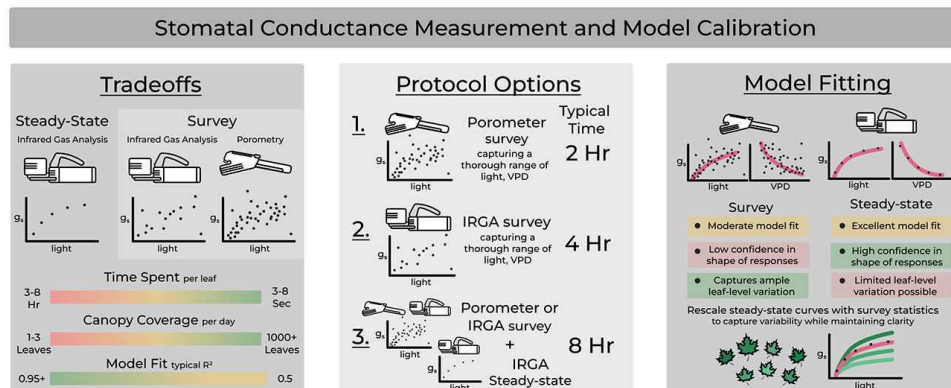
A commonly used approach for stomatal conductance measurement involves conducting survey measurements at ambient conditions, offering a high-throughput means of obtaining stomatal conductance for model calibration. However, this approach is complicated by the disparate time scales of light, heat, and stomatal aperture variation. Stomatal aperture, a result of biological and mechanical processes (Buckley et al., 2003), takes on the order of tens of minutes to hours to reach steady-state in response to environmental perturbations, while leaf light fluxes and leaf temperatures vary with time scales orders of magnitude faster (Chazdon and Pearcy, 1991; Leigh et al., 2006). This means that stomata are often not in equilibrium with their environment, which creates a mismatch between the measured stomatal conductance and the measured environmental conditions at that instant. This temporal mismatch can generate substantial noise in survey measurements, obscuring steady-state relationships.

For single leaves, smooth environmental response curves can be generated by placing a single patch of leaf in a gas-exchange cuvette with microenvironmental control, setting cuvette conditions, and recording the stomatal conductance value after the leaf has reached steady-state equilibrium with the cuvette conditions (typically 10-60 minutes per measurement Buckley and Mott, 2000; Tinoco-Ojanguren and Pearcy, 1993). This is repeated for a range of cuvette conditions to obtain curves describing responses to environmental conditions. While this approach typically provides very smooth stomatal responses and excellent model fits, it is limited by its time-intensive nature, often taking many hours to complete per leaf. As such, representation of leaf-to-leaf spatial variability is limited.

To address these challenges, we present three field-ready protocols for measuring stomatal conductance with model calibration in mind, each tailored to different instrumentation availability and labor hour constraints. The first is the fastest approach, utilizing a porometer for survey measurements, with careful consideration given to minimizing the impact of transient effects. The second uses a portable gas exchange system for survey measurements which is slower and more expensive than a porometer but also measures carbon fluxes and provides point estimates of photosynthesis for stomatal conductance models that incorporate photosynthesis

model predictions. The third uses survey and steady-state measurements in tandem, with the steady-state measurements providing smooth environmental responses, and with the survey measurements capturing leaf-to-leaf physiological variation.

It should be noted that many popular stomatal conductance models also require prediction of net photosynthesis, the parameters of which require separate data for calibration. This protocol focuses on measurement of stomatal conductance only, and gas exchange measurement for photosynthesis model fitting is covered in a separate protocol.



Schematic depiction of options for leaf-level measurement of stomatal conductance for model calibration. Three different protocols are presented, depending on time and instrumentation availability: 1) porometer survey measurements - lowest cost and time requirements, but provide noisier data with less accurate stomatal conductance data; 2) IRGA-based survey measurements - intermediate cost and time requirements, providing noisy data with more accurate stomatal conductance measurement as well as photosynthetic assimilation measurements relevant to some models; 3) hybrid IRGA-based steady-state and porometer (or IRGA) survey measurements - highest cost and time requirements, but most robust parameter estimations.

Attachments



[StomatalConductanceP.](#)

2.1MB



Materials

Specific instrument settings described in these protocols are for the LI-COR instruments listed below. Adaptations may be needed in order to use instruments from other manufacturers.

- LI-600 Porometer/Fluorometer (LI-COR Biosciences, Lincoln, NE, USA)
- (Optional) LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA)

In addition to data collection, we also discuss parameter calibration based on software options given below.

- PhoTorch (<https://github.com/GEMINI-Breeding/photorch>)
- (Optional) PhoTorch Studio (<https://github.com/photorch-studio>)
- (Optional) Other plant physiological model fitting library (e.g., *plantecophys* of Duursma, 2015)
- (Optional) Any programming language with a non-linear least squares fitting algorithm implemented

Materials and Conditions

1 Ambient Conditions Checklist

These protocols are intended to be applied on plants in the field, and certain ambient weather conditions will limit their feasibility. Model calibration will be most effective with ambient light reaching $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (or the typical maximum diurnal light intensity of a specific location) and leaf vapor pressure difference ranges of at least 10–30 mmol mol^{-1} . This can most consistently be achieved on a day with:

1. Full sun and no or minimal cloud coverage
2. Minimal wind
3. A daily high air temperature of at least 18°C (65°F)

A rule of thumb to keep in mind is the LI-6800 chamber conditions can achieve roughly $\pm 10^\circ\text{C}$ from the console temperature, and console temperature can reach 5–15°C above ambient air temperature when heated in full sun.

Preferred conditions for performing these protocols typically occur between mid-morning and solar noon, as it contains a large range of conductances from the still-shaded leaves to those exhibiting diurnally maximal conductance in full light. Capturing this wide range of leaf-level conditions is important for robust model calibration.

These protocols could be adapted to perform measurements in the laboratory, but this will likely limit the range of possible environmental conditions. In this case, it would be desirable to measure plants growing in the brightest light and warmest air temperature possible. Similarly, the maximum light intensity used throughout the protocols may be adapted for plants that receive much lower typical maximum light intensities.

2 Plant Material

These protocols were developed to be used on intact, fully mature, healthy leaves of broadleaf plant species. The underlying stomatal parameters extracted from this data are expected to exhibit seasonal, phenological, and interannual change, so a modeler should be aware of when calibration data was collected and for what period in time model parameters will be used in simulation or for comparison. Similarly, whole-plant water status is very likely to have an effect on stomatal parameters in most species, so if measurements are made under well-irrigated conditions, they are likely to be applicable only under similar conditions. Concomitant time series of stem water potential measurements may be helpful in addition to protocols given here if feasible.

3 Equipment

Specific instrument settings described in these protocols are for the LI-COR instruments listed below. Adaptations may be needed in order to use instruments from other manufacturers.

- LI-600 Porometer/Fluorometer (LI-COR Biosciences, Lincoln, NE, USA)
- (Optional) LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA)

4 Model Parameter Calibration Software

In addition to data collection, we also discuss parameter calibration based on software options given below.

- PhoTorch (<https://github.com/GEMINI-Breeding/photorch>)
- (Optional) PhoTorch Studio (<https://github.com/photorch-studio>)
- (Optional) Other plant physiological model fitting library (e.g., *plantecophys* of Duursma, 2015)
- (Optional) Any programming language with a non-linear least squares fitting algorithm implemented

Initial Instrument Set-up

- 5 Prior to use in respective protocols, the chosen instruments will need to be set up, typically once per day or per measurement session. The set-up procedure described is not meant to be exhaustive and represents only typical set-up tasks performed before each measurement session. Users should consult manufacturer documentation for a complete guide on instrument set-up.

6 LI-600 Porometer Initial Set-up

- 6.1 Power on the instrument.
- 6.2 Select the Configuration to be used. The “Auto gsw+F” or equivalent custom configuration with flow rate set to High and operated in Auto Mode is recommended.
- 6.3 Allow the Match to complete.

7 LI-6800 Initial Set-up

- 7.1 Connect head (handheld unit) via cable and tube.
- 7.2 Check head has correct chamber aperture size installed. The largest aperture should be used that can be consistently covered entirely by sampled leaves.
- 7.3 Connect H₂O Add column, and ensure there is plenty of water in the column.
- 7.4 Install new CO₂ cartridge.
- 7.5 Check the desiccant level. If there is less than 1/2 orange Sorbead® (or blue Drierite®) left, replace it.
- 7.6 Remove red cover from light sensor.
- 7.7 Power on the instrument.
- 7.8 Aperture size: On the *Start Up* → *Chamber Setup* tab, press the button for the appropriate aperture size installed based on the prior step above.
- 7.9 Dynamic Equations: On the *Start Up* → *Chamber Setup* tab, check the *Add Dynamic Equations* box. Then, on the *Constants* → *Gas Exchange* tab, check the *UseDynamic* box.
- 7.10 Perform CO₂ and H₂O point matching: on the *Measurements* tab, press *Match IRGAs*. In the upper right, press *Auto* under *Matching at a single reference point*. When both the CO₂ and H₂O lights turn green simultaneously, matching is complete. Double-check the *Time Since Last Match* field to verify success. If the match failed (i.e., time since last match is more than a few seconds ago), repeat the matching procedure.
- 7.11 H₂O range matching: *Constants* → *Range Match*. Press *H₂O Range Match* → *Start*. Select the Normal (5 min) match and set Flow_s/Flow_r = 1.13.
- 7.12 Perform H₂O dynamic tuning: *Constants* → *Dynamic*. Ensure H₂O control is on. *Utilities/Tests* tab → choose *H₂O Test Current* → *Start*

Protocol 1: Non-steady-state (survey) LI-600 porometer

- 8 Repeat the procedure below 100-200 times based on leaves randomly sampled throughout the canopy. The goal is to sample the wide range of spatial and environmental variability throughout the canopy. As described in more detail below, a critical objective is to choose leaves that appear to have consistently been in their present light environment (either sunlit or shaded) for at least 20 minutes or more.
- 8.1 Choose a fully-expanded 'sunlit' or 'shaded' leaf patch that appears healthy, has no visible water on the surface, and appears to have been in its current condition for the prior 20 minutes or so (try to avoid sunflecks or shadeflecks). A 'sunlit' leaf patch is one that may be angled in any direction but is in direct sunlight with no shading (as such its light level may be significantly less than 2000 PPFD due to its angle relative to the sun). A 'shaded' leaf patch is one whose direct line of sight to the sun is occluded by other leaves.
- 8.2 Carefully clamp the chamber onto the leaf patch.
 - Be sure the leaf patch covers the entire chamber aperture and avoid veins or other areas of non- smooth texture.
 - When clamping the chamber onto the leaf, do not change the leaf angle. It is often tempting to angle the instrument to provide a better view of the instrument display or to better support its weight - this must be avoided.
 - Ensure that the instrument's light sensor is not shaded or differently sunlit than the surface being measured (this can easily occur given the offset between the chamber and light sensor position). The goal is for the measurement of the light level to be representative of the natural leaf, which requires the sensor to be at the same angle and ambient light level as the leaf prior to measurement.
- 8.3 Take the measurement by pressing the measure button, and ensuring an automatic re-match window was not activated. In the event that the re-match window occurs, remove the leaf, and allow the match to occur before proceeding with further measurements.
- 8.4 Repeat on a variety of leaves that are under different light conditions. It is important to capture variation in both light and leaf vapor pressure difference where possible, while still maintaining conditions of (1). Even at solar noon, variation in light will occur due to variation in leaf angles and occlusion.
- 8.5 Note that model fit performance will generally scale hyperbolically with the number of leaf samples. About 200 leaves may be needed to reach a 5% relative error in parameter extraction, and 75 leaves for 20% error tolerance. A suggested target would be 150 leaves measured across the span of 10:00-13:00.

Protocol 2: Non-steady-state (survey) LI-6800 gas exchange

- 9 Pre-measurement set-up specific to this protocol: Remove chamber light source so the clear-top chamber is exposed to ambient light. Set instrument parameters as shown in the table below. Open a log file with an appropriate name to indicate it stores survey measurements.

	Group	Parameter	Value
	Flow	Flow	Auto - 500 $\mu\text{mol/s}$
		Overpressure	Auto - 0.1 kPa
		Pump (speed)	Auto
	H ₂ O	On/Off Toggle	Off
	CO ₂	CO _{2_r}	430 $\mu\text{mol/mol}$
		Soda Lime - Scrub	Auto
	Fan	Fan Speed	5,000 rpm
	Temperature	On/Off Toggle	Off
	Light	-	-

Table 1. Instrument settings for LI-6800 (LI-COR Biosciences, Lincoln, NE, USA) to be used for survey measurements of stomatal conductance. Note: Temperature and H₂O control are set to "Off" so as to result in chamber air being as equivalent to ambient air as possible and to avoid instrument control feedback from causing additional instability in the system and slowing down survey measurement speed. We believe H₂O_r is the most accurate on-board proxy for the water vapor mole fraction of the ambient air surrounding the leaf before it was clamped, which is used to calculate VPD_{leaf}. Alternatively, if humidity control is desired, or if H₂O_r is set to zero for potentially increased stability, the presumably less accurate but available *console* H₂O value can be used to compute VPD_{leaf}. The IRGA-based H₂O_r is expected to be more accurate than the non-IRGA-based *console* H₂O.

- 9.1 The measurement procedure is identical to Procedure 1 above for the LI-600 (Sect. 8), except for the measurement logging step (Step 8.3 above).

The chamber of the LI-6800 is much larger than that of the LI-600 and requires more time for the air to thoroughly mix and reach equilibrium. In this time, however, the leaf temperature will begin to rapidly drift from its original, undisturbed, in-situ temperature.

To capture both the in-situ leaf temperature in addition to all other variables that require the chamber to reach equilibrium before a measurement can be made, a "double-log" for each measurement is recommended. Upon immediate clamping of the leaf in the chamber, and ensuring good contact between the leaf and the thermocouple inside the chamber, take a measurement by pressing the physical *Log* button on the head handle or the digital *Log* on the console screen. Confirm the measurement was taken with the chime of the instrument and the increment of the log counter shown in the top right of the console screen. Then, wait until chamber conditions have reached equilibrium (less than a 1% change from a median value of *gsw*, usually 30-60 seconds but not more), and press *Log* again to take another measurement. Again, ensure the log was recorded as it is important to be sure that every odd log was an "immediate" measurement and every even log was a corresponding "equilibrated" measurement for post-processing.

Protocol 3: Non-steady-state survey and Steady-state Gas Exchange

10 Pre-measurement set-up specific to this protocol: Install the LI-6800 chamber head light source. Initially set chamber conditions to those in the table from Step 9.

11 Steady-state light response measurement

Then, complete a steady-state light response and a vapor pressure deficit response using chamber settings in the tables below. It is recommended to complete a light response on a healthy, sunlit, fully-mature leaf, then complete a vapor pressure deficit response on a second independent leaf of equivalent status to minimize the unknown effects of a single leaf patch being in a chamber for too long. For each of the response curves, perform the following steps below.

	Group	Parameter	Value
	Flow	Flow	Auto - 500 $\mu\text{mol/s}$
		Overpressure	Auto - 500 $\mu\text{mol/s}$
		Pump (speed)	Auto
	H2O	RH _{air}	60%
	CO2	CO2 _r	Auto - 500 $\mu\text{mol/s}$
		Soda Lime - Scrub	Auto

	Group	Parameter	Value
	Fan	Fan speed	5,000 rpm
	Temperature	Tair	25C
	Light	Head light source setpoint (Qin)	2,000 $\mu\text{mol}/\text{m}^2/\text{s}$

Table 2. General steady-state measurement instrument settings for LI-6800 (LI-COR Biosciences, Lincoln, NE, USA) to be used for steady-state measurements of stomatal conductance. Qin, Tair, and/or RH_air are changed from this reference state when carrying out the response measurements given in Tables 3 and 4.

	Qin ($\mu\text{mol}/\text{m}^2/\text{s}$)	Tair ($^{\circ}\text{C}$)	RH
	2,000	25	60
	1,600		
	1,200		
	600		
	100		
	0		

Table 3. Chamber settings for steady-state light response measurements: head light source (Qin), air temperature in $^{\circ}\text{C}$ (Tair) and chamber relative humidity (RH_air). Each of the 6 environment combinations are repeated in sequence on a single leaf, waiting until *stomatal steady-state* at each light level.

	Qin ($\mu\text{mol}/\text{m}^2/\text{s}$)	Tair ($^{\circ}\text{C}$)	RH
	2,000	25	60
		30	60
		30	40
		35	40
		35	20

Table 4. Chamber settings for steady-state VPD response measurements: head light source (Q_{in}), air temperature in °C (T_{air}) and chamber relative humidity (RH_{air}). Each of the 5 environment combinations are repeated in sequence on a single leaf, waiting until *stomatal steady-state* at each VPD level.

- 11.1 Choose a fully-expanded 'sunlit' leaf that appears healthy, has no visible water on the surface, and is on a 'sunlit' side of the plant or tree crown during the measurement period if possible. Clamp the chamber on the center of the leaf carefully, making sure the entire chamber area is covered by the leaf. Use an appropriate chamber aperture size for a given species.
- 11.2 Open a log file. Name it according to the chamber condition chosen. (For each subsequent chamber condition (T, RH, Q), create a new log file. This will minimize the chance of crashing or freezing due to large log files).
- 11.3 In the Measurements tab, create three graphs. One with gsw versus obs or time. One with gsw versus Q_{in} . One with gsw versus VPDleaf.
- 11.4 Go to the Programs tab, and from the drop-down menu select Autolog. Set the logging interval to 5 sec, and set the runtime to a large number (e.g., 9999 sec). Press start to run the program.
- 11.5 Let stomata reach steady-state. Watch the gsw versus time plot in the Measurements tab until they appear to have roughly reached steady state (less than a 2% percent change across 3-5 minutes or a 5% change after 15 minutes, typically following a sigmoidal response to new conditions). This may take 30 mins or more. Zoom out on the graph as far as possible to see these responses at an appropriate scale. It will often appear as though the stomata have reached steady-state after a few minutes. However, in most cases, this is only instrument stability and not true stomatal steady-state.

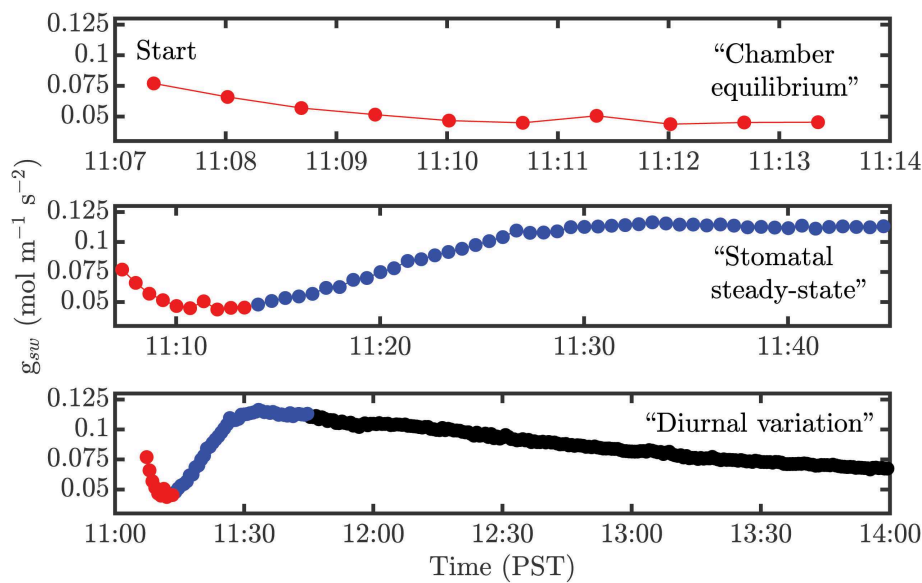


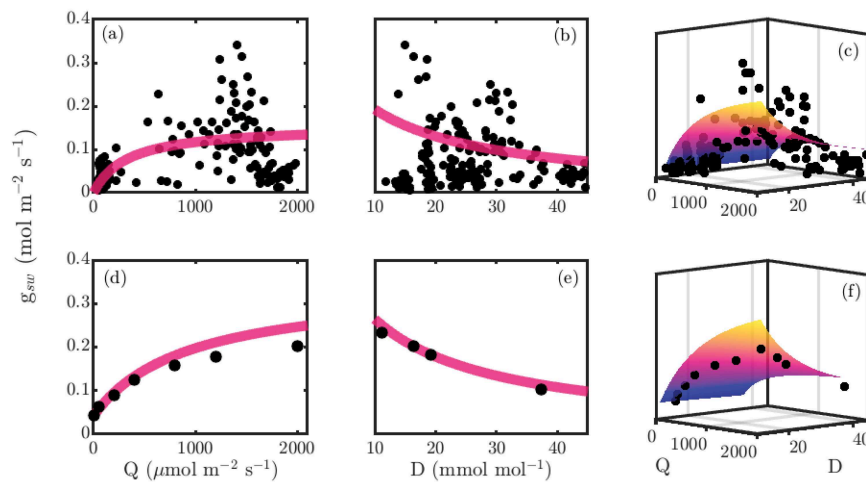
Illustration of the three temporal phases observed in “steady-state” stomatal measurements. A single Texas red oak leaf (*Quercus buckleyi*) was held in a LI-COR LI-6800 chamber from 11:00 to 17:00 PST under a constant PPFD of $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$, $T_{\text{air}} = 22^\circ\text{C}$ and $RH = 35\%$. Within minutes the chamber reaches equilibrium, allowing repeatable readings, but stomata require approximately 25 min to attain true “stomatal steady-state.” Over the subsequent hours, a slower diurnal drift—likely driven by plant water status or circadian regulation—reduces stomatal conductance by about 50%

- 11.6 Run through the light or vapor pressure deficit response settings given in Tables 3 and 4, respectively. Create a new log file for each set of conditions, and wait for stomatal steady-state at each condition. Observe the g_{sw} versus light and VPD plots to make sure measurements are reasonable. If using one LI-6800, run through the light response on one leaf, and the VPD response on another leaf after. If using two LI-6800's, run the light response on one leaf and the VPD response on another leaf, parallel in time.

Data Analysis

12 Processing Survey Data

Survey measurements need little processing prior to model fitting. Data may occasionally need filtering to remove outliers with unreasonably high or low, or null values. In some cases, a negative stomatal conductance is measured (computed), which has no physical interpretation. A judgment call may be made to replace those with zeros or a prescribed minimum conductance, or to remove them entirely.



An example of non-steady-state stomatal conductance data as measured in Protocols 1-3 is shown in (a)-(c), and steady-state data as measured in Protocol 3 is shown in (d)-(f). Stomatal conductance is shown plotted against two primary drivers of stomatal aperture change, photosynthetic flux density (Q ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) in (a), (d) and leaf-to-air water vapor mole fraction difference (D ; mmol mol^{-1}) in (b), (e), and fit to a model (Buckley, Turnbull, and Adams 2012) of Q and D shown in (c), (f). Steady-state data typically provides an excellent model fit (here, adjusted $R^2 = 0.99$ and $RMSE = 0.0063 \text{ mol m}^{-2} \text{s}^{-1}$) while non-steady-state data provides a decent model fit (adjusted $R^2 = 0.54$ and $RMSE = 0.0444 \text{ mol m}^{-2} \text{s}^{-1}$)

For LI-6800 survey data, VPD_{leaf} is calculated by the instrument using the cuvette air's water vapor mole fraction H_2O_s . For a more representative value of the ambient air surrounding the leaf before it was put into the cuvette, H_2O_r can be used to manually re-calculate VPD_{leaf} using

$$VPD_{\text{leaf}} = SVP_{\text{leaf}} - H_2O_r \cdot \frac{Pa}{1000} ,$$

where SVP_{leaf} is the saturation vapor pressure of the leaf (kPa), and Pa is the ambient air pressure (kPa). Each of these is reported in a separate column of the LI-6800 data file. If humidity control is used, H_2O_r may no longer reflect ambient conditions and can be substituted for the reported console H_2O , which is assumed to be less accurate than the IRGA-based H_2O_r measurement but more reflective of ambient humidity if H_2O_r deviates from ambient for purposes of cuvette humidity control.

13 Processing Steady-State Data

The continuous logs of stomatal conductance during steady-state responses need to be processed into single point estimates for model fitting. This can be achieved by taking the end point (not recommended) or an average of a window on the tail end of the time series (recommended). The appropriate window length can vary for each continuous log depending on the amount of variation about an apparent mean. A common feature is oscillatory stomatal behavior with species-specific frequency. If observed, the window length should exceed any apparent oscillation wavelength (and ideally be as close to some integer multiple as possible) so as to cancel positive and negative amplitudes and arrive at an unbiased mean. Logs with a flat plateau may need a window length as little as 20 seconds, while logs with apparent variation may need a window as large as several minutes. Other logs may have unique, less understood features that need to be carefully inspected and a 'steady-state' value chosen by hand.

14 Model Fitting

The objective of these protocols is to obtain data for fitting models of stomatal conductance in order to extract model parameters. To demonstrate the differences between survey and steady-state measurements for model calibration, we fit data collected by Protocols 1 and 3 to the semi-empirical model of Buckley, Turnbull, and Adams (2012), which is a function of photosynthetic flux density (Q ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and leaf-to-air water vapor mole fraction difference (D ; mmol mol^{-1}). The non-steady-state data collected in Protocols 1-3 is much faster to collect but contains much more scatter about the model surface due to transient stomatal dynamics and leaf-to-leaf physiological variation compared to the steady-state data collected in Protocol 3 of a single leaf. For the steady-state data, adjusted $R^2 = 0.99$ and $RMSE = 0.0063 \text{ mol m}^{-2} \text{s}^{-1}$, while non-steady-state data resulted in an adjusted $R^2 = 0.54$ and $RMSE = 0.0444 \text{ mol m}^{-2} \text{s}^{-1}$; these are typical results for each of these fits. Data collected with the above protocols can be fit with a Python library, PhoTorch, following the latest instructions found in the documentation (<https://github.com/GEMINI-Breeding/photorch>), or with a simple graphical user interface, PhoTorch Studio (<https://github.com/photorch-studio>). Similarly, fitting can be performed in many other languages using off-the-shelf non-linear least squares regression algorithms. An example is given below in base Python and using PhoTorch below.


```
import pandas as pd
from scipy.optimize import curve_fit
from numpy import inf

# Buckley-Turnbull-Adams model (Buckley et al., 2012)
def BTA(X, Em, i0, k, b):
    Q, D = X
    return Em * (Q + i0) / (k + b * Q + (Q + i0) * D)

# Load and preprocess data (from an LI-600 file)
df = pd.read_csv("file.csv", skiprows=1).drop(index=0) # Drop
units row
a = 0.85 # Absorbed fraction of ambient PPFD (unitless)
Q = pd.to_numeric(df["Qamb"])*a # Absorbed PPFD (umol/m2/s)
P = pd.to_numeric(df["P_atm"]) # Air pressure (kPa)
D = pd.to_numeric(df["VPDleaf"])*1000/P # VPD (mmol/ mol)
gsw = pd.to_numeric(df["gsw"]) # (mol/m2/s)

# Set parameter initial guesses, lower and upper bounds
p0 = [10, 100, 5, 1e4]
bounds = ([0, 0, 0, 0], [inf, inf, inf, inf])

# Fit and extract model parameters
p, _ = curve_fit(BTA, (Q, D), gsw, p0=p0, bounds=bounds)
Em, i0, k, b = p

#### OR, with PhoTorch

from photorch import stomatal
import pandas as pd
import torch

# Read in the data and initialize the fitter
df = pd.read_csv("file.csv", skiprows=1).drop(index=0) # Drop
units row
df = df.apply(pd.to_numeric, errors="coerce") # Set all values to
numeric
df['CurveID'] = 1 # Add a CurveID to the dataset
data = stomatal.initscdata(df) # Import the dataset into PhoTorch
model = stomatal.BMF(data) # Select the model

# Fit and extract model parameters
fitresult = stomatal.fit(model, learnrate = 0.5, maxiteration =
20000)
```

```
fit = fitresult.model
Em = fit.Em.item()
i0 = fit.i0.item()
k = fit.k.item()
b = fit.b.item()

# Print parameters
print("Em ", Em)
print("i0 ", i0)
print("k ", k)
print("b ", b)
```

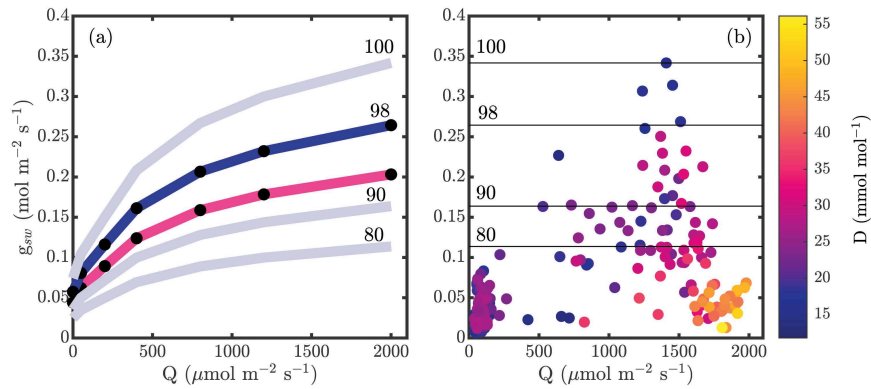
15 Optional rescaling of steady-state fits with survey statistics

The steady-state data exhibits less model error, allowing for greater confidence in the shape of the light and vapor pressure deficit responses for the measured leaf, but there is no expectation that the chosen leaf is representative of other leaves of that individual plant, cultivar or species. If the chosen leaf happens to be an outlier, despite appearing healthy and fully mature, it would compromise the representativeness of derived model parameters. Survey data across many leaves, on the other hand, implicitly captures leaf-to-leaf physiological variation, but this (along with transient dynamics) leads to difficulty determining reliable response curve shapes. The strengths of the survey and steady-state data obtained in tandem using Protocol 3 can be leveraged in order to use the smooth response curve shapes from the steady-state data, but rescaling the magnitude to be representative of canopy-scale variability.

Rescaling of each steady-state stomatal conductance, $g_{sw,ss,i}$ for all i data points, can be performed using the following transformation prior to model fitting:

$$g_{sw,ss,i} = g_{sw,ss,i} \cdot \frac{\text{percentile}(G_{sw,nss}, p)}{\max(G_{sw,ss})},$$

where $\text{percentile}(G_{sw,nss}, p)$ returns the p -th percentile of the set of non-steady-state stomatal conductance, $G_{sw,nss}$, and $\max(G_{sw,ss})$ returns the maximum of the set of steady-state stomatal conductance data, $G_{sw,ss}$. A rescaling to the 98th percentile is shown in the figure below, but many other statistics may be considered. The steady-state maximum stomatal conductance is measured at $2000 \mu\text{mol m}^{-2} \text{s}^{-1} Q$ and around $15 \text{ mmol mol}^{-1} D$, so for most applications it makes sense to consider the survey points within this range for the rescaling.



An example of rescaling steady-state stomatal conductance with survey statistics. A steady-state stomatal light response often takes hours to produce for one single leaf, limiting its ability to provide confidence in representativeness for the many leaves of a plant canopy. In (a), a measured light response (pink) was rescaled (blue) using statistics derived from non-steady-state point measurements in (b). Percentiles are marked in both subplots, and the 98th percentile used here to rescale the steady-state response, but many different statistics could be used depending on application.

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