

1 Factors influencing the measurement of assimilation and stomatal conductance with the 2 LI-COR 6400XT gas exchange system

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11
12 **Abstract:** Although CO₂ and H₂O exchange rates are often measured in experiments as indicators of
13 physiological plant responses these “gas exchange” measurements are prone to large experimental error.
14 Gas exchange equipment and technology have improved greatly over the past two decades which supports
15 scrutinizing current issues of experimental error in measuring plant photosynthesis and stomatal
16 conductance. This report shows results of a greenhouse experiment with the goal of identifying lesser
17 understood sources of experimental error and variation in measurements with the LI-COR 6400XT gas
18 exchange system. A variety of plant types were used to encompass differing species variation. We found
19 significant sources of experimental error in 1) the time for initial adjustment when placing a leaf in the
20 leaf chamber 2) the time-of-day when measuring 3) leaf age 4) having the chamber window full vs.
21 partially full with leaf tissue 5) using a leaf chamber environment that greatly diverges from the whole
22 plant environment 6) differing degree of experimental error depending upon plant species. A situation
23 with multiple contributors to error would result in useless gas-exchange data. Recommendations for
24 minimizing these experimental errors are given.

25 26 27 **Introduction:**

28 Although CO₂ and H₂O exchange rates are often measured in experiments as indicators of
29 physiological plant responses these “gas exchange” measurements are prone to large
30 experimental error. Certainly many actual factors influence photosynthesis and stomatal
31 conductance, such as plant water and nutrient status, but there are additional methodological
32 factors that contribute to error. To some degree each researcher must identify and minimize
33 experimental error in their specific project, leading to an extensive “learning curve” for gas
34 exchange measurements. Currently, there is a shortage of practical information to help users
35 reduce measurement variability. A literature search shows that reports on factors influencing leaf
36 gas exchange are somewhat dated (Long et al. 1996; Long & Bernacchi 2003). Manufacturers of
37 gas exchange systems, such as LI-COR Biosciences (Lincoln, Nebraska, USA) typically provide
38 instruction and documents that assist the researcher in gathering reliable gas exchange data.
39 Although gas exchange equipment and technology have improved greatly over the past two
40 decades there continue to be multiple sources of experimental error and uncertainty in gas
41 exchange data. Prudent researchers typically utilize these well-understood factors:

- 42 1) Measure recently fully expanded leaves to reduce variability due to leaf age.
43 2) Take measurements during the peak hours of plant activity.
44 3) Set the gas-exchange leaf chamber conditions near “ambient”; temperature, CO₂,
45 humidity etc.
46 4) Use a non-limiting light intensity.

47 Improvements in the technology of leaf gas exchange equipment support taking another look at
48 these issues. This report shows results of a greenhouse experiment with the goal of identifying
49 lesser understood sources of experimental error and variation in measurements with the LI-COR
50 6400XT gas exchange system. A variety of plant species were used to include much of the
51 existing variation in leaf size, photosynthetic systems (C₃ & C₄), and phylogeny .
52 Recommendations toward balancing error with the practicality of experiments are included. The
53 critical parameters of net CO₂ assimilation (hereafter “assimilation”; A) and stomatal
54 conductance (Gs) were measured. Leaf level assimilation is a fairly straight-forward
55 measurement of CO₂ flux (corrected for water vapor dilution), whereas stomatal conductance is a
56 calculated parameter, requiring measurement of leaf transpiration rate, leaf temperature, and
57 atmospheric conditions (temperature, water vapor, pressure) (Ball, Woodrow & Berry 1987). .
58 We quantified the change in assimilation and stomatal conductance resulting from various
59 manipulations of plant and leaf chamber conditions. Following these manipulations, we assumed
60 leaf-level equilibration when A and Gs stabilized to the chamber conditions. This was ascertained
61 by plotting A and Gs against time using the 6400XT software- .
62 The experiment attempted to answer the following questions:

- 63 1) How long does it take to achieve equilibration after changing leaf chamber conditions to each
64 of light, CO₂, humidity and temperature?
- 65 2) Does time to equilibration vary greatly in well-watered vs. water-stressed plants?
- 66 3) How much variability is typical with changing time of day?
- 67 4) To what degree does leaf age affect gas-exchange?
- 68 5) Is there an effect of having the leaf chamber only partially filled, as is often the case when
69 measuring plants with small leaves (even when measurements are normalized by leaf area)?
- 70 6) How independent is leaf-level functioning (within the leaf chamber) from whole plant level
71 functioning?
- 72 7) Do these factors vary between monocots and dicots or between C₃ and C₄ species?

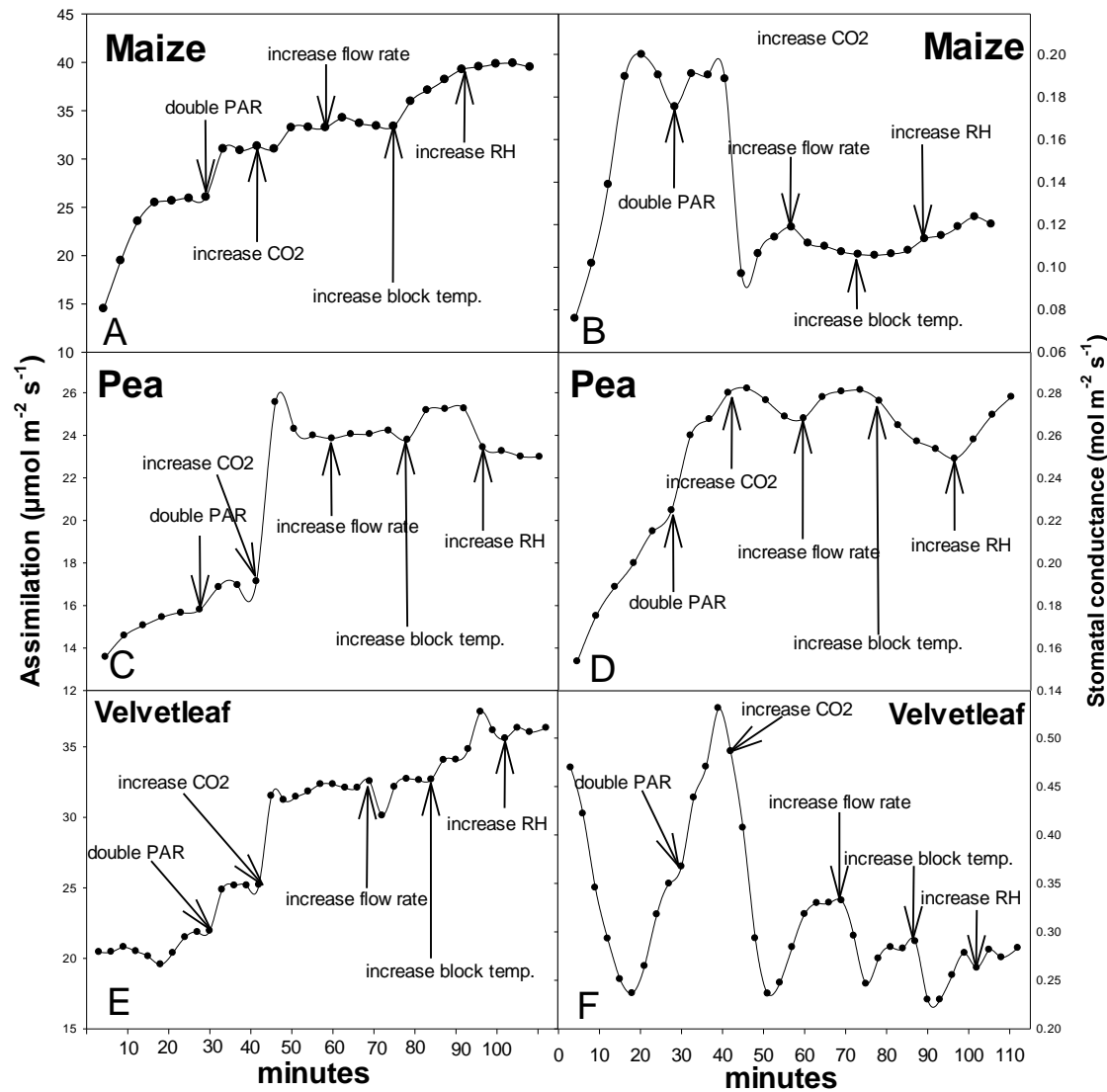
73 74 **Methods:**

75 We utilized the LI-COR Biosciences 6400XT steady-state gas-exchange system (2010 model)
76 with the narrow leaf chamber and red/blue light source (6400-02B). Calculations were performed
77 in the 6400XT software (OPEN version 6.2.5). When leaves were too small to fill the chamber
78 we carefully measured leaf area and used the LI-COR recalculation spreadsheet.

79
80 Four species were grown from seed: Maize (*Zea mays* - “Flint corn”) a wide-leaved C₄-type
81 photosynthesis monocot; *Pascopyrum smithii* (Western wheatgrass) a narrow-leaved C₃
82 monocot; Pea (*Pisum sativum* - “Lastons #9) a small-leaved C₃ dicot and *Abutilon theophrasti*
83 (Velvetleaf) a large-leaved C₃ dicot. Three replications of each were sown in a commercial
84 potting soil and grown in a greenhouse under a 12-hour photoperiod (700 to 1900 hours) with
85 28/20 °C day/night, 800 μmol m⁻² s⁻¹ photosynthetic active radiation (PAR), 25% relative
86 humidity (RH) and 400 ppm [CO₂]. Day-length was increased during this winter experiment
87 using high pressure sodium and metal halide lamps. Plants were irrigated daily and received

88 weekly fertilizer supplements (Dyna_gro All Pro 7-7-7; Richmond, CA). Seeds were sown on
89 October 24, 2017 and measurements began November 24, 2017, at which time most plants were
90 of suitable size for measurements (except Western wheatgrass). LI-COR 6400 XT measurements
91 were performed on the same greenhouse bench as the plants were growing. Standard 6400XT
92 startup and calibration protocol was carefully followed. Frequent “matching” of the reference
93 and sample analysis cells was performed, especially when the CO₂ concentration was altered.
94 The leaf thermocouple was touching the leaf for all measurements, and therefore, the energy
95 balance method for leaf temperature was not used. Leaf chamber CO₂ concentration and RH
96 were controlled with the “reference” method (incoming conditions rather than outgoing). Leaf
97 temperature was controlled by the block method, such that the temperature of the aluminum
98 chamber surrounding the leaf (i.e., block) was adjusted to keep leaf temperature confined to a
99 narrow range. Generally, measurements needed to be taken within a short time window during
100 each day. Considering that equilibration times were often significant, this usually allowed for
101 only one measurement per species on each day.

102
103 **Trial one:** How long does it take to achieve equilibration after changing leaf chamber conditions
104 to each of light, CO₂, humidity and temperature? Unfortunately, Western wheatgrass plants were
105 quite small when other species were ready to measure and therefore were not included in trial
106 one. All plants were well watered. Measurements were performed from about 1000 to 1400
107 hours, bracketing mid-day (preliminary tests showed that Maize was slow to reach daily peak gas
108 exchange rates and therefore Maize was always measured last). Mid-sections of fully mature
109 leaves of each species were clamped in the chamber at starting conditions similar to the
110 greenhouse: 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, chamber block temperature of 24 °C, 450 ppm CO₂ and RH
111 of ca 25%. The chamber window was full (6 cm² leaf area) for all three species. Once A and Gs
112 had equilibrated to the chamber starting conditions the conditions were changed and monitored
113 for a new equilibrium in response to 1) doubling the PAR, 2) doubling the [CO₂], 3) increasing
114 the block temperature by 8 °C, and 4) doubling the RH. We also tested the response to changing
115 the chamber flow rate. Although flow rate is generally not considered a critical parameter, we
116 desired to know if leaf physiology was altered at differing flow rates. Data was recorded every 3
117 minutes and A and Gs were plotted to determine when equilibrium to chamber conditions had
118 been reached. Only one replication of each species was measured for trial one.



119
 120 **Figure 1.** The response of Maize, Pea and Velvetleaf assimilation (A, C, E) and stomatal
 121 conductance (B, D, F) to changes in the leaf chamber environment (photosynthetically active
 122 radiation (PAR), CO₂ concentration, flow rate, chamber temperature and relative humidity (RH)
 123 using the LiCor 6400XT gas exchange system.

124
 125 **Results:** Maize was slow to equilibrate to the starting conditions, taking about 12 minutes
 126 (Figure 1A & B). The 6400XT achieved adjustments in chamber environment very quickly for
 127 all parameters, and therefore was not a delaying factor in the analyses. The Maize response to
 128 increasing PAR and increasing CO₂ were very fast (less than 3 minutes) (Figure 1 A & B). The
 129 G_s response to doubling CO₂ was marked and quickly detected (Figure 1B). As predicted there
 130 was little response to increased flow rate. Assimilation slowly increased with increasing leaf
 131 chamber temperature, taking about 12 minutes to resume equilibration. Surprisingly, G_s had little
 132 response to higher temperature, and therefore higher vapor pressure deficit (VPD). Similarly,
 133 there was little response to increased RH, and therefore lower VPD (Figure 1A & B).

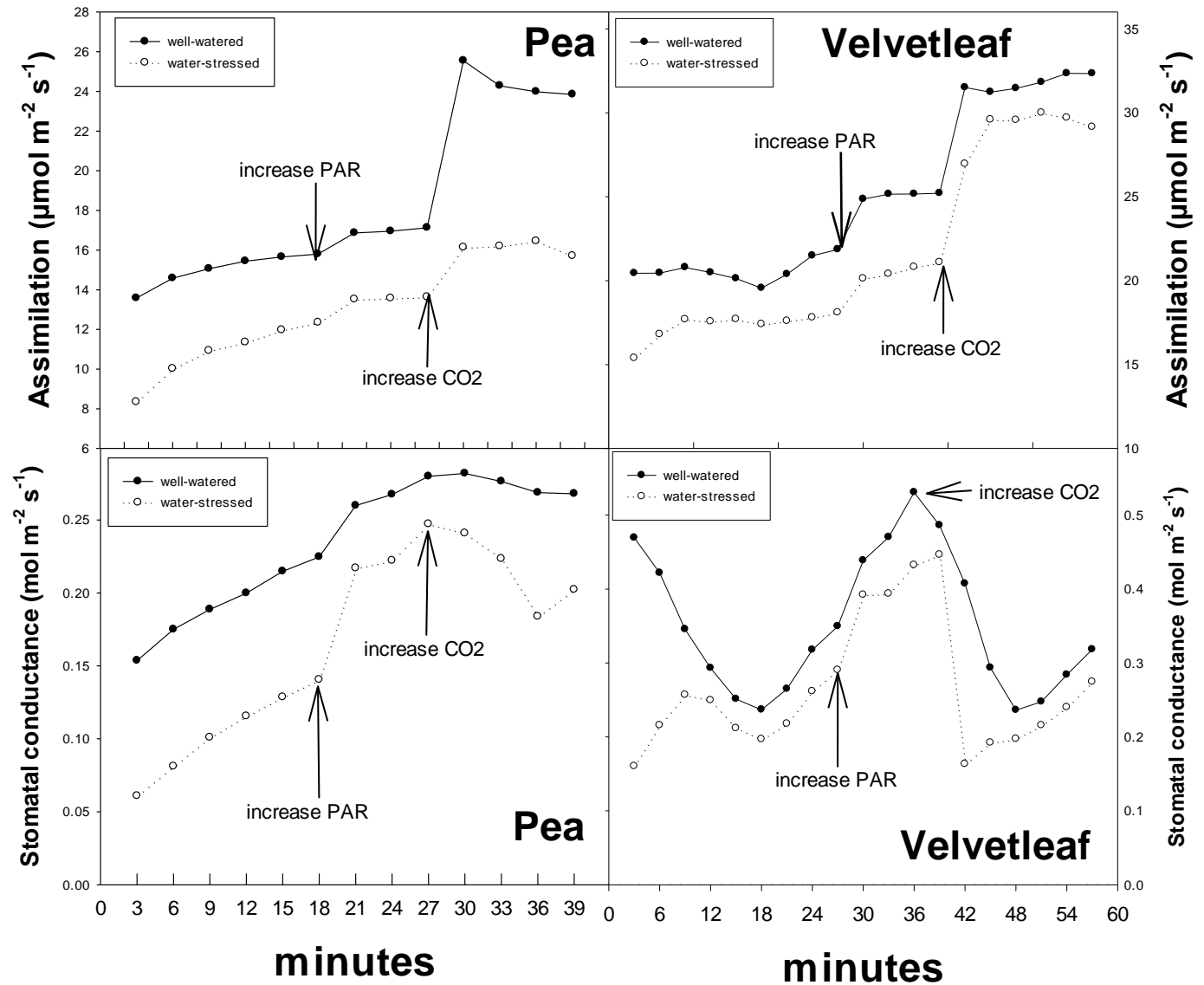
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135 Pea leaves were also slow to equilibrate to starting conditions, taking about 15 minutes (Figure
136 1C & D). Similar to Maize, equilibration to changes in chamber environment were quite fast;
137 about 3 minutes for an increase in PAR, temperature, RH and [CO₂]. There was a small change
138 in G_s in response to higher flow rate, but not A (Figure 1 C & D). The stomatal conductance
139 response to warmer and higher RH chamber conditions was slower than the assimilation
140 response. In general assimilation equilibrated more quickly than stomatal conductance.

141
142 Velvetleaf was very slow to equilibrate to starting conditions, particularly in stomatal
143 conductance, taking about 21 minutes to reach a steady-state (Figure 1E & F). Assimilation
144 responses to chamber changes were fast but G_s responses were slow and erratic. In particular,
145 Velvetleaf G_s reacted to a change in flow rate that was not fully compensated for in the
146 calculations.

147
148 Despite a chamber environment very similar to the growth environment, all three species
149 required a long time to reach full equilibrium to the leaf chamber (ca 16 minutes). It is typical to
150 report allowing 5 minutes or less for initial equilibration to a gas-exchange chamber (LICOR
151 6400XT manual). Our results suggest up to a 25% error if data are logged at 5 minutes. Our test
152 shows that subsequent changes to chamber conditions are fast, re-equilibrating in about 3
153 minutes. Furthermore, G_s generally equilibrated more slowly than A, particularly for VelvetLeaf,
154 where G_s exhibited a slow and erratic equilibration. Stomatal changes to vapor pressure may be
155 particularly slow (Turner et al. 1984). VelvetLeaf also seemed to be affected by a change in flow
156 rate, which in theory is completed adjusted for in the calculations.

157
158 **Trial two:** Does equilibration time vary greatly in well-watered vs. water-stressed plants? Trial
159 two was performed at the end of the study period but is presented here for comparison with trial
160 one. Pots were allowed to dry to about 20% of their saturated weight. This seems extreme but the
161 potting soil used had a very large water holding capacity. This degree of soil drying was just
162 above the visible wilting point for all species. In order to shorten the measurement duration, only
163 chamber PAR and [CO₂] were altered. Surprisingly, Maize A and G_s rates were nearly the same
164 as in well-watered plants, suggesting that we did not achieve the desired water stress, and
165 therefore we do not report data for these species here.



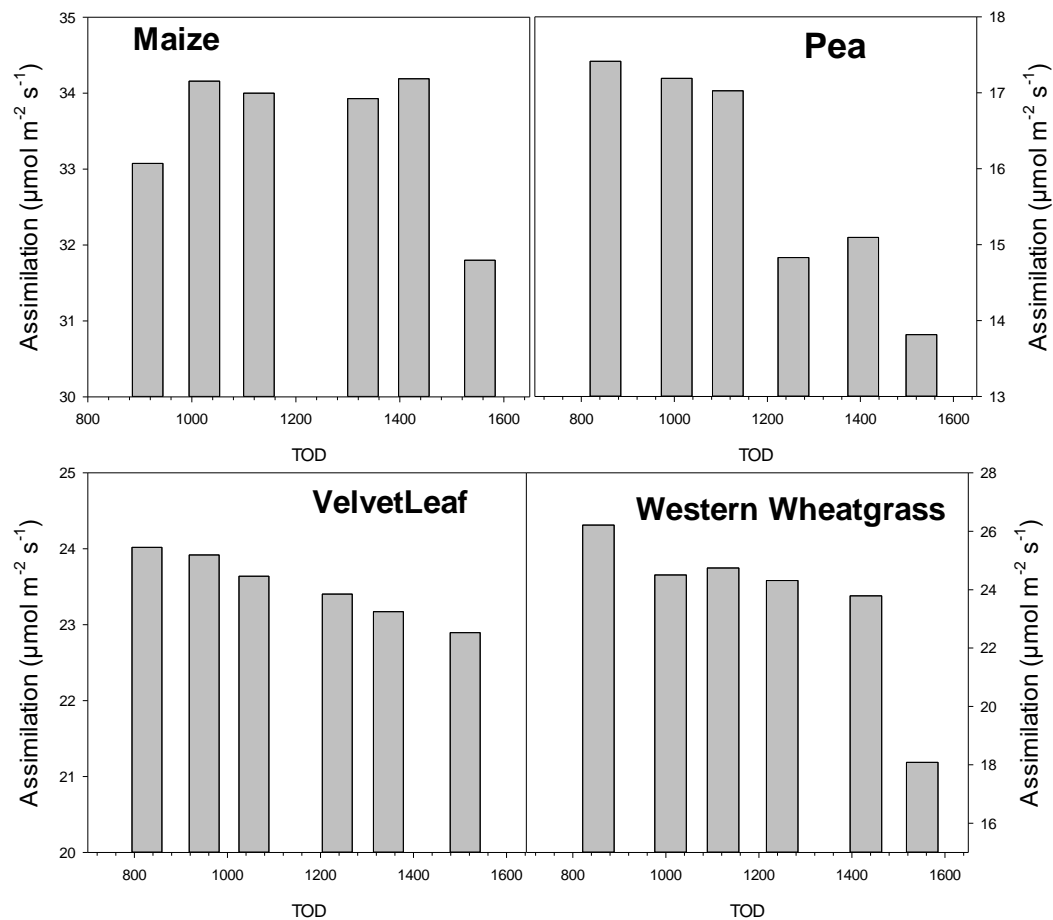
166
 167 **Figure 2.** The response of Pea and Velvetleaf assimilation and stomatal conductance to changes
 168 in photosynthetically active radiation (PAR) and CO_2 concentration in well-watered vs. water-
 169 stressed plants using the LiCor 6400XT gas exchange system.

170
 171 **Results:** Pea leaves of water-stressed plants responded to chamber changes in PAR and $[\text{CO}_2]$ in
 172 parallel to well-watered plants, albeit with lower A and Gs rates (Figure 2A & C). As with well-
 173 watered plants, stressed Pea plants were quite slow to equilibrate to starting conditions and were
 174 still adjusting slightly after 18 minutes. There was a slightly different response in Gs to
 175 increasing $[\text{CO}_2]$ in water stressed plant, with a slow decrease in Gs which was not seen in well-
 176 watered plants. However this is an expected response to higher $[\text{CO}_2]$ in most plants (Figure 2C).
 177

178 Water-stressed Velvetleaf responded similarly to changing chamber conditions as the well-
179 watered plants (Figure 2B & D). Again, it required a long time for Gs to equilibrate to starting
180 conditions (ca 20 minutes), however the initial slope of the adjustment was opposite in water-
181 stressed plants (Figure 2D).
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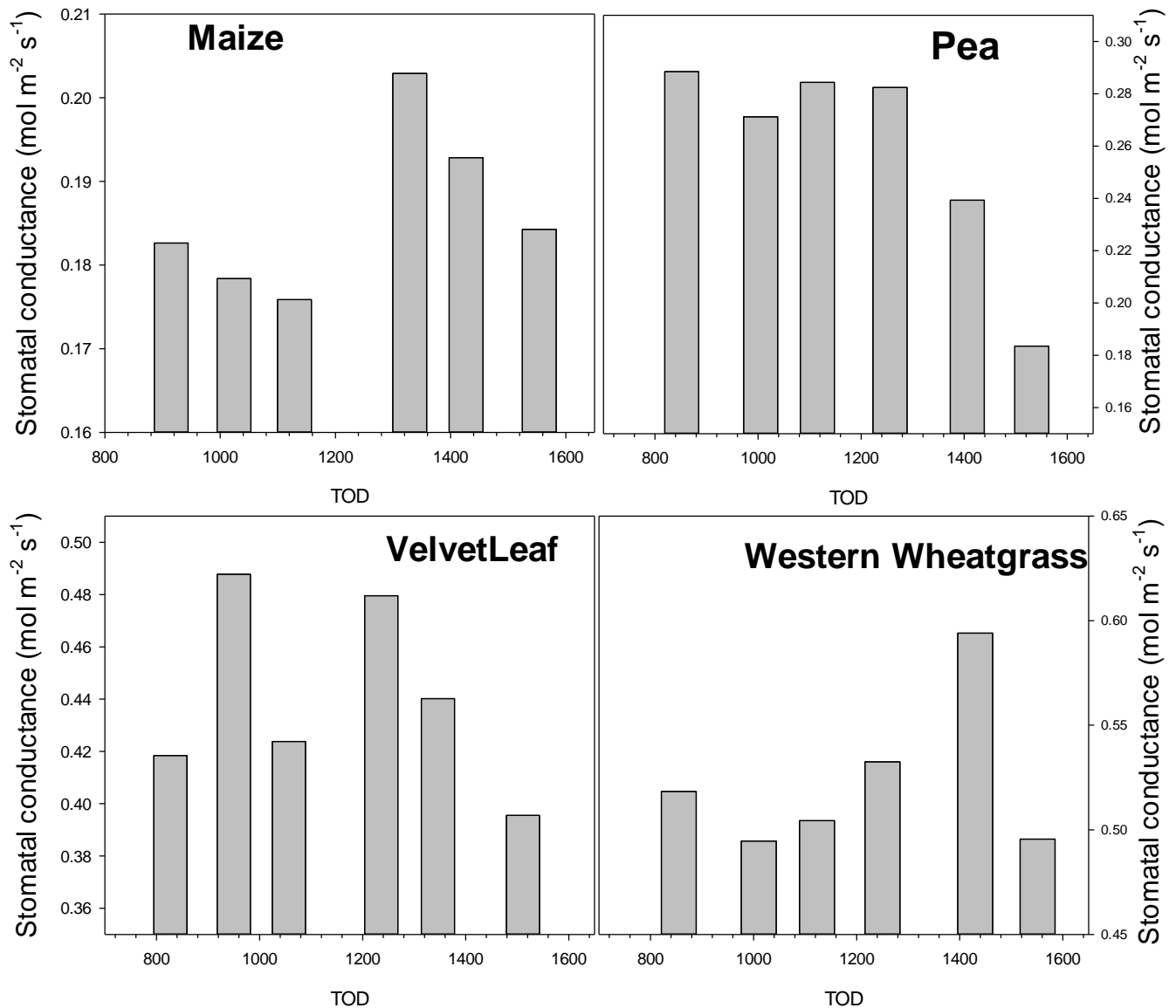
183 Although we have a limited dataset, it appears that plants subjected to mild water-stress will
184 equilibrate to changes in leaf chamber environment in a similar way as well-watered plants. The
185 initial equilibration to the leaf chamber will be quite slow, but subsequent changes in
186 environment will be compatible with a 5 minute logging interval. Different species and degrees
187 of water stress could have contrary results to ours.
188

189 **Trial 3:** How much variability is typical with time of day? Leaves of all four species were
190 measured early in the photoperiod and the leaf area that was in the chamber was marked and
191 returned to the chamber at six different times during the photoperiod. Therefore the exact section
192 of the leaf was measured over the course of a day. Plants were kept well-watered throughout.
193 Chamber conditions were similar to the greenhouse environment except that saturating light was
194 used: $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, 450 ppm $[\text{CO}_2]$, 25 °C and 25% RH during all measurements.



195
196 **Figure 3.** The influence of time-of-day of on the assimilation rate of Maize, Pea, Velvetleaf and
197 Western wheatgrass using the LiCor 6400XT gas exchange system.

198 **Results:** Assimilation decreased late in the photoperiod for all species but there were differences
199 among species (Figure 3). Maize A did not decrease until about 10 hours into the photoperiod
200 (Figure 3A). Maize also did not achieve peak A until after 0900 hour. Pea assimilation decreased
201 shortly after midday (Figure 3B). Velvetleaf declined slightly over the course of the day (Figure
202 3C). Western Wheatgrass A did not decline until about 10 hours into the photoperiod (Figure
203 3D).



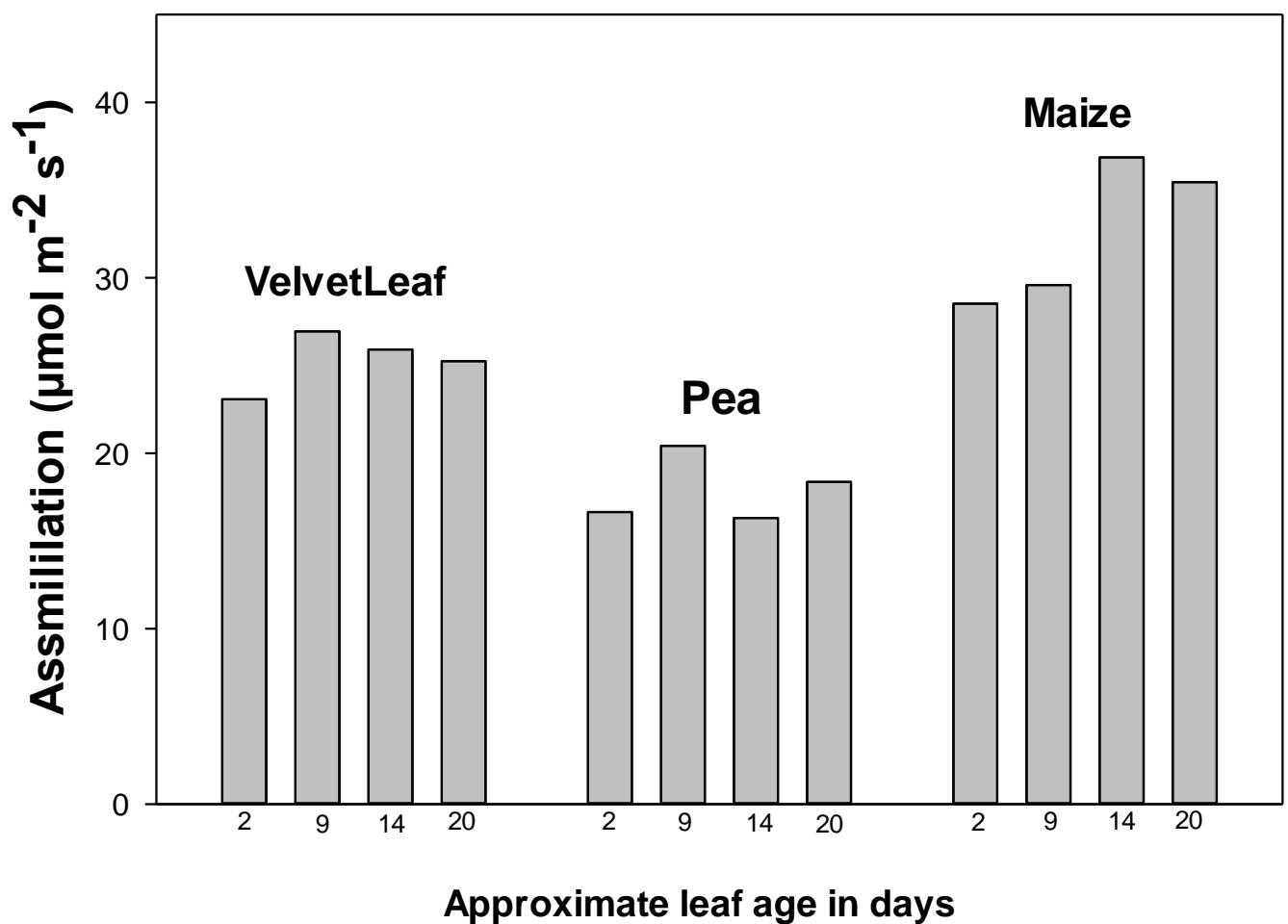
204 **Figure 4.** . The influence of time-of-day on the stomatal conductance of Maize, Pea, Velvetleaf
205 and Western wheatgrass using the LiCor 6400XT gas exchange system.
206

207 Stomatal conductance rates over the photoperiod were less consistent than assimilation (Figure
208 4). Pea Gs behaved similarly to A with a strong decline about 10 hours into the photoperiod
209 (Figure 4B), however other species had more erratic Gs rates. The striking finding was that Gs of

210 Maize was lower in the morning vs after midday, although only by ca 15% (Figure 4A; note the
211 small range on the Y axis). In general, consistent measurement of Gs was more difficult than A.

212 **Trial 4:** To what degree does leaf age affect gas-exchange? For this question leaves were labelled
213 with the date when they became fully expanded (as plant growth progressed). When there was a
214 20 day range in leaf age these leaves were measured under chamber conditions similar to the
215 greenhouse environment as in Trial 1. Western wheatgrass was not measured due to difficulty in
216 determining leaf age. We report only assimilation data (Figure 5), but note that stomatal
217 conductance responded similarly in all cases.

218



219

220 **Figure 5.** The influence of leaf age on the assimilation rate of Velvetleaf, Pea and Maize using
221 the LiCor 6400XT gas exchange system.

222 **Results:** Under greenhouse conditions with plentiful irrigation and nutrients, leaf age, ranging by
223 about 18 days, had minor effects on assimilation rates (Figure 5). However it is noteworthy that
224 Maize A did not peak until leaves were over 9 days old. This is somewhat contrary to the
225 perception that gas-exchange rates are highest in young and recently expanded leaves (Long et

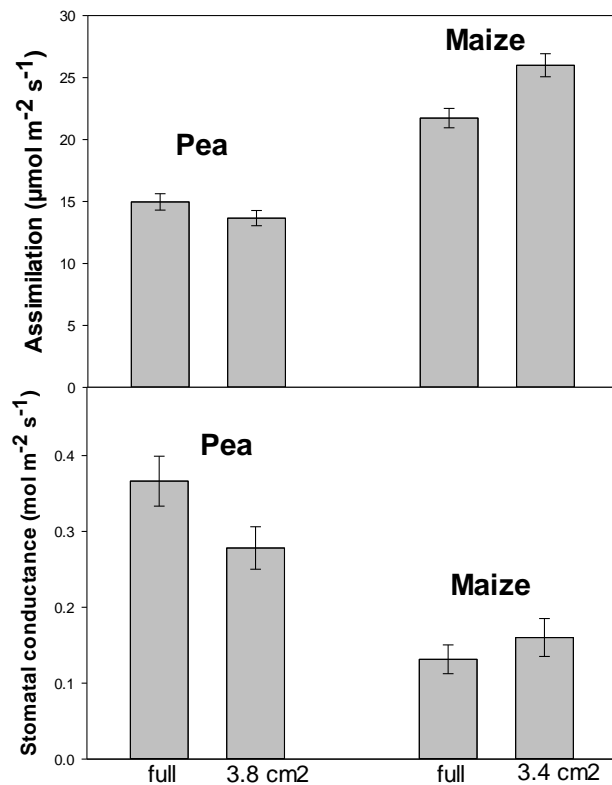
226 al. 1996). However this again may be a response to the general low light greenhouse conditions
227 during the winter and C₄ photosynthesis of Maize.

228

229 **Trial 5:** Is there an effect of having the leaf chamber window only partially filled (even with
230 measurement of leaf area)? Many wild and cultivated species exhibit leaves that are smaller than
231 the chamber window of most gas exchange systems. This situation results in only a partially
232 filled chamber, and subsequently, requires the recalculation of both A and G_s. However, it
233 remains uncertain if the air flow through a partially filled chamber (relative to a full chamber)
234 differs, or results in meaningfully different measurements of A or G_s. At the very least
235 researchers are often encouraged to standardize the amount of leaf in a partially filled chamber
236 window. Trial 5 was a simple test of this issue using Maize and Pea leaves. This trial was
237 performed on two replicates for each of Maize and Pea.

238 For Maize, a large leaf was chosen that filled the entire chamber (6 cm²). This leaf was allowed
239 to fully equilibrate, and A and G_s were recorded. Then, a slit was made along the length of the
240 leaf to create a more narrow section of leaf, which was then quickly returned to the chamber. The
241 chamber area filled by the cut Maize leaf was determined from leaf width (measured with digital
242 calipers) and chamber length.

243 For Pea, a large leaf that filled the chamber window was measured at full equilibrium, then a
244 smaller leaf that was nearby on the same stem was measured. The smaller leaf area was
245 measured on the LiCor area meter (3000A).



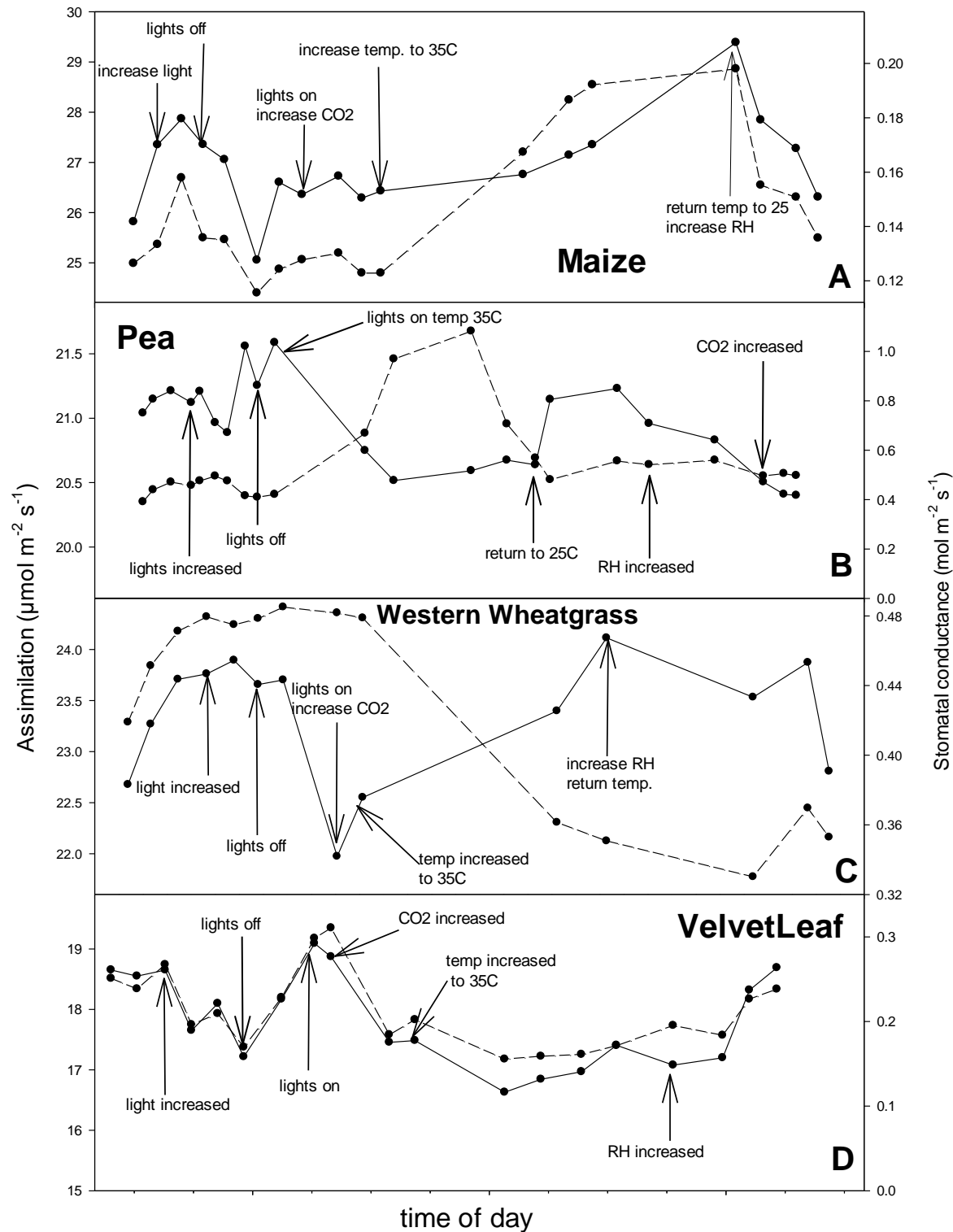
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247 **Figure 6.** The influence of a full vs. partially full leaf chamber window on assimilation and
248 stomatal conductance in Pea and Maize using the LiCor 6400XT gas exchange system.

249 **Results:** Pea leaves that only partially filled the chamber window had slightly lower A and Gs
250 (Figure 6). We note that these differences could have resulted from random variation across
251 leaves, although the large and small leaves were very close on the stem. Maize leaves that
252 partially filled the chamber had slightly higher A and Gs rates, and this was measured on the
253 very same leaf tissue. It is possible that the cut edge of the Maize leaf could have influenced this
254 test, however the cut surface area was very small compared to the intact leaf surface area. It is
255 also possible that air circulation around both sides of the leaf was slightly different in the
256 partially filled chamber and that this had a small effect on the measurements.

257

258 **Trial 6:** How independent is leaf-level functioning (within the leaf chamber) from whole plant
259 level functioning? To have confidence in gas exchange measurements the leaf portion that is in
260 the leaf chamber must be responding primarily to the chamber environment. Chamber conditions
261 that diverge from the conditions of the whole plant, such as measurement at elevated CO₂, will
262 hopefully have the desired influence on the leaf section in the chamber, rather than the conditions
263 the remainder of the plant is exposed to (outside the leaf chamber). One must also assume that
264 the chamber is not leaky to the external environment (Rodeghiero et al. 2007). Trial 6 tested the
265 independence of the leaf portion in the chamber and the whole plant. Whole plants were placed
266 in controlled environment growth chambers (Conviron BDR16, Winnipeg, Manitoba, Canada)
267 and the growth chamber conditions were altered under steady 6400XT leaf chamber conditions.
268 The starting growth chamber conditions were 25C, 30% RH, 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and 420ppm
269 CO₂. These starting conditions were changed to 35 °C, 80% RH, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, zero
270 PAR (lights off), and 750 ppm CO₂. Note that these manipulations were not always in the same
271 order for each species, although we do not expect this affect the responses. 6400XT leaf
272 chamber conditions were 25C, 30% RH, 1000 PAR and 420 CO₂ and were unaltered.
273 Because the growth chamber had to be closed during these tests, the 6400XT console was
274 viewed through a window in the growth chamber door and when equilibrium was reached the
275 data were quickly recorded, the growth chamber door was closed and the next growth chamber
276 manipulation began. Growth chamber changes in PAR, [CO₂] and RH were quite fast, however it
277 took about 15 minutes for the growth chamber to increase by 10 C.
278



279

280 **Figure 7.** The influence of changes in the whole plant environment (growth chamber light level,
281 CO₂ concentration, temperature and relative humidity) on assimilation (solid line) and stomatal
282 conductance (dashed line) while holding the leaf chamber environment steady in Maize, Pea,
283 Western wheatgrass and Velvetleaf using the LiCor 6400XT gas exchange system.

284 **Results:** Maize single leaf gas exchange was strongly influenced by a 10 degree increase in the
285 temperature of the whole plant; both A and Gs increased even though the leaf chamber
286 temperature remained at 25C (Figure 7A). Maize also exhibited a small but temporary response
287 to both increasing and decreasing the light on the whole plant. There was no effect of whole
288 plant CO₂ environment, nor to increasing the relative humidity.

289 Pea leaves had an especially strong and positive Gs response to an increase in whole plant
290 temperature (note Y axis range), however this response was temporary (Figure 7B). Pea
291 exhibited a small but noticeable decrease in assimilation in response to increased whole-plant
292 temperature. Similar to Maize, there appeared to be very small and temporary responses to
293 changing light, [CO₂] and RH.

294 Western Wheatgrass exhibited an increase in assimilation, but a decrease in stomatal
295 conductance in response to increasing whole plant temperature (Figure 7C). There was little
296 effect of changing whole plant PAR, CO₂ nor RH.

297 Assimilation in VelvetLeaf declined slightly in response to higher [CO₂] and higher whole plant
298 temperature, however, there was much variation in these measurements (Figure 7D). An increase
299 in whole plant RH elicited a rather large increase in A and Gs. Assimilation and stomatal
300 conductance tracked well in Velvetleaf.

301 Single leaf gas-exchange was affected by divergence in whole plant environment in all four
302 species. In particular, leaves held at constant climate conditions within the 6400XT chamber
303 exhibited meaningful changes in functioning when whole-plant temperature was increased by 10
304 °C. Maize and Western wheatgrass assimilation exhibited a positive response to increasing whole
305 plant temperature, whereas the two dicotyledons exhibited negative responses. In general,
306 divergence in whole plant vs leaf section functioning were very small for changes in [CO₂], PAR
307 and RH. However, VelvetLeaf and Western Wheatgrass exhibited small negative responses to
308 increasing whole plant [CO₂].

309 Finally, we ask, do measured responses differ between monocots and dicots, or between C₃ and
310 C₄ plants? Certainly, what we can interpret from this small set of species is quite limited. Of note
311 however, is that Maize stomatal conductance was very low during the morning hours. This could
312 be due to the relatively low light environment in the greenhouse in the winter, since C₄ plants
313 perform well under conditions of high heat and irradiance. There was also some indication that
314 VelvetLeaf responded more erratically than the other species when leaf chamber conditions
315 where changed. In particular, Gs exhibited much variation in this species (see Figure 3 & 7). We
316 suspect that this result may have arisen due to the very thin leaves of this species. VelvetLeaf
317 also responded to increasing flow rate, i.e., the 6400XT did not appear to fully normalize A and
318 Gs for flow rate. This suggests that boundary layers may have been dissimilar between Velvetleaf
319 and the other species.

320 Not surprisingly, results varied with species, emphasizing that researchers should perform a
321 thorough trial run of gas exchange measurements on their species of interest. Field measurements
322 will be very difficult to standardize for leaf age, full vs. partially full chamber, whole plant vs.
323 chamber conditions, plant water and nutrient status and time of day influences, all adding to the
324 high variability in gas exchange data.

325 **Recommendations:**

326 Table 1 summarizes the potential error in assimilation for each of the investigated factors. There
327 was some evidence that the experimental error may have been higher in stomatal conductance
328 measurements. Certainly, a worse-case scenario involving multiple factors could easily result in
329 useless gas-exchange data. In addition to these method concerns there are significant sources of
330 error in actual experiments due to plant phenology, variable soil water and nutrition, intra- and
331 interspecific genetic variation etc. Additionally, unique stem and leaf morphologies may add
332 another layer of difficulty, resulting in difficult clamping and sealing of leaves (Rodeghiero et al.
333 2007). Researchers must always attempt to balance known and unknown experimental error with
334 the practical aspects of gathering gas-exchange data. Limited personnel, time and even weather
335 can often dominate protocol decisions. We recommend that researchers conduct as thorough
336 preliminary tests as possible prior to gathering important gas-exchange data. At the very least,
337 standardization of gas-exchange protocols is important for: 1) time of day, 2) temperature, 3) leaf
338 age, 4) leaf size in the chamber, 5) criteria for determining equilibrium, and 6) divergence from
339 whole plant conditions. Thorough preliminary tests and data analysis will likely reduce the
340 amount of undesired variation in gas-exchange measurements. Increasing the number of
341 replications per study group should add additional confidence to gas-exchange data.

342

343 **Table 1.**

Influencing factor on net CO ₂ assimilation	Potential error (%)
Allowing only 5 minutes for a leaf to equilibrate to initial chamber conditions	up to 25%
Allowing only 5 minutes for a leaf to equilibrate to a change in chamber conditions	very little
Measuring plants late in the photoperiod	up to 30%
Measuring leaves of different ages	up to 22%
Measuring leaves which differentially fill the chamber window	up to 17%
Measuring leaves at markedly different temperature than the whole plant	up to 12%

344

345 **Literature citation:**

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360 *Plant, Cell and Environment*: 1006-1022.