Use of the response of photosynthesis to oxygen to estimate mesophyll conductance to carbon dioxide in water-stressed soybean leaves

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ABSTRACT

Methods of estimating the mesophyll conductance \( g_m \) to the movement of CO\(_2\) from the substomatal airspace to the site of fixation are expensive or rely upon numerous assumptions. It is proposed that, for C\(_3\) species, measurement of the response of photosynthesis to \([O_2]\) at limiting \([CO_2]\), combined with a standard biochemical model of photosynthesis, can provide an estimate of \( g_m \). This method was used to determine whether \( g_m \) changed with \([CO_2]\) and with water stress in soybean leaves. The value of \( g_m \) estimated using the \( O_2 \) response method agreed with values obtained using other methods. The \( g_m \) was unchanged over the tested range of substomatal \([CO_2]\). Water stress, which decreased stomatal conductance (\( g_s \)) by about 80%, did not affect \( g_m \), while the model parameter \( V_{\text{Cmax}} \) was reduced by about 25%. Leaves with \( g_s \) reduced by about 90% had \( g_m \) values reduced by about 50%, while \( V_{\text{Cmax}} \) was reduced by about 64%. It is concluded that \( g_m \) in C\(_3\) species can be conveniently estimated using the response of photosynthesis to \([O_2]\), at limiting \([CO_2]\), and that \( g_m \) in soybean was much less sensitive to water stress than \( g_s \), and was somewhat less sensitive to water stress than \( V_{\text{Cmax}} \).

Key-words: drought; internal conductance; oxygen inhibition.

INTRODUCTION

With recent evidence that the conductance of the pathway for CO\(_2\) movement from the intercellular airspace to the site of fixation inside the chloroplast during photosynthetic CO\(_2\) fixation, termed mesophyll conductance \( g_m \), is functionally significant and is not simply a physical diffusive conductance (reviewed in Flexas et al. 2008; Warren 2008b), there has been renewed interest in how it may limit photosynthesis in different species (Warren & Adams 2006), with various stresses (Centritto, Loreto & Chartzoulakis 2003; Galmes, Medrano & Flexas 2007), and affect the acclimation of photosynthesis to environment (e.g. Singsass, Ort & DeLucia 2003; Ethier et al. 2006; Yamori et al. 2006; Bunce 2008). Unfortunately, methods of estimating \( g_m \) are expensive and not readily available to most researchers and/or rely upon assumptions that are difficult to prove. Among the many methods of estimating \( g_m \) (reviewed in Warren 2006), there are three basic types commonly used: discrimination among isotopes of carbon during photosynthesis, combined fluorescence and leaf gas exchange measurements, and estimates based on the curvature of the slope of the response of photosynthesis to substomatal CO\(_2\) concentration (\( C_i \)). The instrumentation required for online measurements of carbon isotope discrimination is expensive and not available to most researchers, and estimation of \( g_m \) with this method relies upon assumptions about discrimination by non-photosynthetic processes (Evans et al. 1986). The method using the curvature of \( A \) versus \( C_i \) curves (Ethier & Livingston 2004) may not be appropriate if \( g_m \) varies with the \([CO_2]\), as found by Flexas et al. (2007) and During (2003). The two types of fluorescence estimates, the ‘constant J’ method (Bongi & Loreto 1989) and the ‘variable J’ method (Di Marco et al. 1990) each have limitations (discussed in Harley et al. 1992), sometimes disagree significantly (Bunce 2008) and may depend on which leaf surface the fluorescence signal is viewed from (Lichtenthaler, Buschmann & Knapp 2005; Bunce 2008). The interpretation of fluorescence signals in drought-stressed plants also remains uncertain (Osmond, Kramer & Luttge 1999). It is proposed that, for C\(_3\) species, the measurement of the response of photosynthesis to \([O_2]\), for example from 2 to 21% \( O_2 \), to limiting \([CO_2]\), combined with a standard Farquhar-type biochemical model of C\(_3\) photosynthesis (Farquhar, von Caemmerer & Berry 1980), can provide an estimate \( g_m \) that avoids many of these issues. As an example, this method was used to determine whether \( g_m \) changed with \([CO_2]\) and with water stress in soybean leaves.

THEORY OF THE METHOD

The method relies on the fact that \( O_2 \) and \( CO_2 \) compete for RuBp at ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisco), and this competition determines the rate of net photosynthesis as long as neither substrate is saturating. The sensitivity of \( CO_2 \)-limited photosynthesis to a change in
finite gm can be found such that A at low [O2] can be pre-
provided that A is limited by either VCmax or J at both [O2].

(Cc). As evident in the preliminary data for Fiskeby
calculated from any pair of measurements of A at two [O2],
J estimated from the data at 21% [O2] also adequately fit
[O2], because of the greater [CO2] sensitivity of A at low
and hence, the predicted value of A more at low than at high
predicted from A at high [O2] with a single value of VCmax or J.

late A at 2% [O2], the calculated rates exceeded measured
versus Ci curves measured at 21% [O2] (filled symbols). Furthermore, the predicted values of A
when limited by VCmax (solid lines) or by J (dashed lines) are
shown based on parameterization of the photosynthesis model
with the values of A measured in 21% [O2]. Cc was calculated
using a constant value of gm of 0.275 mol m$^{-2}$ s$^{-1}$.

[O2] thus provides information on the [CO2] at Rubisco
(Cc). As evident in the preliminary data for Fiskeby soybean,
when values of VCmax and J estimated from the A
versus Cc curves measured at 21% [O2] were used to calcu-
late A at 2% [O2], the calculated rates exceeded measured rates
over the whole range of Cc values (Fig. 1). When a
finite value of gm was assumed, the new values of VCmax and J
estimated from the data at 21% [O2] also adequately fit the
data at 2% [O2] (Fig. 1). It is not necessary to assume that
the same value of gm occurs at all Cc, because gm can be
calculated from any pair of measurements of A at two [O2],
provided that A is limited by either VCmax or J at both [O2].
At a given value of Cc, a finite value of gm would lower Cc,
and hence, the predicted value of A more at low than at high
[O2], because of the greater [CO2] sensitivity of A at low
[O2]. Thus, a unique combination of higher VCmax or J and
finite gm can be found such that A at low [O2] can be predicted
from A at high [O2] with a single value of VCmax or J.

The procedure is illustrated in Table 1. Firstly, a value of
VCmax (or J) is found, which fits the observed A at high [O2]
(21% in this example) at the observed Cc. If the predicted
value of A at low [O2] (2% in this example) exceeds the
observed value, an arbitrary estimated value of gm is chosen
and used to calculate Cc at 21% [O2] and find the new VCmax
(or J) value that fits A at 21% [O2] at that Cc. The new
model value of A at 2% [O2] is then compared with the
observed value at the Cc at 2% [O2]. If the modelled value
of A at 2% [O2] is less than the observed value, then the
estimate of gm is too low, and vice versa (Table 1).

When A versus Cc curves at both [O2] are available, one
can readily pick Cc values that meet the criterion that A at
both [O2] are limited by the same model parameter, VCmax or
J, by comparing the observed A versus Cc curves with the
photosynthesis model (Sharkey et al. 2007). Thus, A and Cc
data at two [O2] values at different Cc, values can be used to
determine whether gm changes with Cc. The main assump-
tions of the method are that competition at Rubisco
described by the Farquhar-type C3 photosynthesis model
fully explains [O2] effects on CO2 fixation, and that respira-
tion in the light is unchanged over the [O2] range used.
Implicit here is that gm is not sensitive to [O2], which was
tested by Loreto et al. (1992). A significant effect of [O2] on
alternative electron sinks could potentially impact the
method when used under conditions where assimilation is
limited by electron transport. The importance of this to
estimates of gm has not yet been experimentally addressed.

When complete A versus Cc curves are not available,
correct estimation of gm still depends on A being limited
by the same photosynthetic model parameter at both [O2].
Therefore, A must be measured at two Cc at both [O2], and
the parameter limiting A at each [O2] deduced by compar-
observed responses of A to Cc, with the photosynthesis model.
Substantial errors in estimating gm could occur if
Vmmax limited A at one [O2] level and J limited it at the other
[O2] level, and the change in limitation was not realized in
the analysis. For example, if J limited A at 21% [O2], but
Vmmax limited A at 2% [O2], then assuming J limitation of
both rates would provide an overestimate of gm, or no solu-
tion at all. If it were assumed that the rate at 2% [O2] was
limited by Vmmax, when it was actually limited by J, then
gm would be underestimated. Comparing gm values as a

![Figure 1. Net CO2 assimilation rate (A) in relation to substomatal [CO2] (Cc) or the calculated [CO2] at ribulose 1,5-bisphosphate carboxylase/oxygenase (Cc) for three Fiskeby soybean leaves measured in 2% [O2] (open symbols) or in 21% [O2] (filled symbols). Furthermore, the predicted values of A when limited by VCmax (solid lines) or by J (dashed lines) are shown based on parameterization of the photosynthesis model with the values of A measured in 21% [O2]. Cc was calculated using a constant value of gm of 0.275 mol m$^{-2}$ s$^{-1}$.](image)

<table>
<thead>
<tr>
<th>A measured (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Cc (µmol m$^{-2}$)</th>
<th>[O2] (%)</th>
<th>gm (µmol m$^{-2}$ s$^{-1}$)</th>
<th>A modelled (µmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.0</td>
<td>250</td>
<td>21</td>
<td>infinite</td>
<td>16.0</td>
</tr>
<tr>
<td>25.3</td>
<td>250</td>
<td>2</td>
<td>infinite</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2</td>
<td>0.330</td>
<td>25.9</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2</td>
<td>0.275</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2</td>
<td>0.220</td>
<td>24.4</td>
</tr>
</tbody>
</table>

The value of gm is that where measured and modelled values of A at 2% [O2] are equal. See text for details. Modelled values of A at 2% [O2] for gm values ±20% of the actual value are also given.
function of $C_i$ is a convenient but arbitrary strategy. If $g_m$ varies with $[CO_2]$, it is completely unknown whether it varies with $C_i$, $C_c$ or something else that co-varies with $[CO_2]$.

The precision with which $g_m$ can be estimated for a given uncertainty in $A$ is greater when $V_{C_{\text{max}}}$ limits $A$ than when $J$ limits $A$, since the slope of $A$ versus $C_i$ is much shallower when $J$ is limiting, especially at 2% $[O_2]$ (Fig. 1). For example, $g_m$ estimated at $C_i = 400 \mu mol \cdot m^{-2} \cdot s^{-1}$ in Fiskeby soybean would range from 0.13 to 0.65 mol m$^{-2}$ s$^{-1}$ for a change in $A$ at 2% $[O_2]$ of 1 mol m$^{-2}$ s$^{-1}$. In contrast, when $A$ is limited by $V_{C_{\text{max}}}$, values of $g_m$ are tightly constrained by $A$ (Table 1).

The sensitivity of estimates of $g_m$ to errors in $K_c$ (1 + O/$K_c$), $R_d$ and $G^*$ of the photosynthesis model (Sharkey et al. 2007) were estimated using the data for Fiskeby at a $C_i$ of 250 $\mu$mol $\cdot$ mol$^{-1}$. Errors of ±10% in $K_c$ (1 + O/$K_c$) produced less than a 10% error in $g_m$, and errors of ±10% for $R_d$ produced less than 4% error in $g_m$ (not shown). Errors of ±10% in $G^*$ produced errors in the range of 20 to 25% in $g_m$ (Fig. 2).

**MATERIALS AND METHODS**

Soybean (Glycine max L. Merr. cv. Fiskeby V and Essex) plants were grown one per 20 cm diameter pot in controlled environment chambers with air temperatures of 25 °C, dew point temperatures of 18 °C and 1000 $\mu$mol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) from a mixture of high-pressure sodium and metal halide lamps for 12 h per day. Chamber $[CO_2]$ was kept between 370 and 400 $\mu$mol $\cdot$ mol$^{-1}$ by injecting $CO_2$ or $CO_2$-free air under the control of an infrared $CO_2$ analyser that sampled chamber air continuously. Pots were filled with vermiculite and flushed daily with a complete nutrient solution containing 14.5 mm nitrogen. Water stress was imposed on Essex plants by terminating the application of the nutrient solution. Leaf gas exchange measurements were made on terminal leaflets of third trifoliolate leaves a few days after area expansion was complete.

Leaf gas exchange measurements were made using a LI-6400 portable photosynthesis system (LI-Cor, Inc., Lincoln, NE, USA). Leaf temperature was controlled at 25 °C, and the leaf to air water vapour pressure difference was controlled between 1.2 and 1.4 kPa by manipulating the water vapour pressure of the incoming air stream. Measurements were made on 6 cm$^2$ sections of intact leaflets at a PPFD of 1500 $\mu$mol m$^{-2}$ s$^{-1}$ provided by red and blue light-emitting diodes. Steady-state rates of assimilation (A) in both 2 and 21% $[O_2]$ were recorded at different $[CO_2]$. The 2% $[O_2]$ gas was obtained by blending $N_2$ with air, using mass flow controllers, and air was assumed to be 21% $O_2$. The system software was used to correct the output of the infrared analysers for the background $[O_2]$ and to calculate $C_i$. Gas exchange measurements were conducted inside a controlled environment chamber, in which the water vapour pressure was controlled to match (±0.1 kPa) that inside the cuvette. This was found to eliminate the need to correct for water vapour leakage into or out of the cuvette (Rodeghiero, Niinemets & Cescatti 2007). Corrections for $CO_2$ leakage were made based on the difference between the chamber and cuvette $[CO_2]$, using the manufacturer’s protocol.

Preliminary testing of the method of estimating $g'_i$ was conducted using three Fiskey V plants. Steady-state rates of assimilation (A) in both 2 and 21% $[O_2]$ were measured at 4 $[CO_2]$ from about 250 to 700 $\mu$mol $\cdot$ mol$^{-1}$.

In the water stress experiment with Essex, prior to the estimate of $g_m$, rates of assimilation (A) in 21% $[O_2]$ at a range of $[CO_2]$ were recorded and used to determine whether $V_{C_{\text{max}}}$ or $J$ was limiting at the $[CO_2]$ used to estimate $g_m$. The measurement sequence for water-stressed plants deliberately included a large step decrease in external $[CO_2]$, so that it could be determined whether stomatal reopening caused by the switch to low $C_i$ caused a shift in the A versus $C_i$ curve. Estimates of $g_m$ were then obtained by equilibrating leaflets at the desired $[CO_2]$ in 21% $[O_2]$ until gas exchange rates were constant. The $[O_2]$ of the inlet air stream was then switched to 2% while maintaining the same external $[CO_2]$. The rate of photosynthesis at 2% $[O_2]$ and $C_i$ were then recorded when stable, but before stomatal conductance ($g_s$) responded to the change in $[O_2]$, i.e. within 2 to 3 min. Stomatal conductance increased after a few minutes exposure to 2% $[O_2]$, presumably because $C_i$ was reduced by the increase in A. This increase in stomatal conductance and $C_i$ was used to determine whether A was limited by $V_{C_{\text{max}}}$ or by J at 2% $[O_2]$ by applying the photosynthesis model to the observed increase in A with $C_i$. After leaf gas exchange measurements were completed on a given leaf, water potential was determined using dew point hygrometry (Wescor HR-33T, Wescor, Inc., Logan, UT, USA) on a disc excised from that leaf.

Values of $g_m$ measured at an external $[CO_2]$ of 380 ± 5 $\mu$mol $\cdot$ mol$^{-1}$ were determined for unstressed leaves of Essex, and for leaves measured on the third or on the
fifth day without nutrient solution application. Measurements were made on leaves of six or seven different plants under each stress condition.

In a subset of three unstressed and three severely stressed leaves, the \( g_m \) was also estimated at \( C_i \) values of 150 \( \pm 10 \) and 400 \( \pm 15 \) \( \mu \text{mol} \text{ mol}^{-1} \) [\( \text{CO}_2 \)]. The lower \( C_i \) value was chosen to be high enough that estimates of \( g_m \) were still insensitive to possible changes in respiration in the light with [\( \text{O}_2 \)] (Tcherkez et al. 2008). The upper value of \( C_i \) was chosen because, for some leaves, assimilation rates became insensitive to \( C_i \) at higher \( C_i \) values, which would invalidate the method of estimating \( g_m \) from the \( O_2 \) response of photosynthesis. A possible dependence of \( g_m \) on \( g_s \) in unstressed leaves, which happened to vary by a factor of 3 in \( g_s \) at 380 \( \mu \text{mol} \text{ mol}^{-1} \) [\( \text{CO}_2 \)], was tested by calculating the correlation between \( g_m \) and \( g_s \) among leaves.

A Farquhar-type \( C_3 \) photosynthesis model with updated kinetic parameters (Sharkey et al. 2007) was used to estimate \( g_m \) from \( A \) and \( C_i \) at 2 and 21% [\( \text{O}_2 \)]. This was done separately for each leaf by determining, by trial and error, values for \( V_{\text{Cmax}} \) (or \( J \)) and \( g_m \) that fit the observed rates of \( A \) at both 21 and 2% [\( \text{O}_2 \)] at a given external [\( \text{CO}_2 \)]. Values of \( g_m \) were resolved to the nearest 0.01 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \). The \( V_{\text{Cmax}} \) Values presented are based on \( C_s \), not on \( C_i \). It was assumed that respiration rate did not change with water stress, based on observations of Ribas-Carbo et al. (2005), and we used their value for respiration (0.5 \( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \)) in the photosynthesis model.

**RESULTS**

Water stress progressively reduced both the initial slope of the \( A \) versus \( C_i \) curves and \( A \) at high \( C_i \) (Fig. 3). For stressed leaves measured at low \( C_i \), data obtained before and after \( g_s \) increased at low \( C_i \) fit on the same \( A \) versus \( C_i \) curve. The \( C_i \) at 380 \( \mu \text{mol} \text{ mol}^{-1} \) external [\( \text{CO}_2 \)] averaged about 290 \( \mu \text{mol} \text{ mol}^{-1} \) for unstressed leaves [mean leaf water potential (LWP) −0.74 MPa], 200 \( \mu \text{mol} \text{ mol}^{-1} \) in moderately stressed leaves (mean LWP −1.41 MPa) and 180 \( \mu \text{mol} \text{ mol}^{-1} \) in severely stressed leaves (mean LWP −1.86 MPa). At these \( C_i \) values, \( A \) was always limited by \( V_{\text{Cmax}} \) at both [\( \text{O}_2 \)]. At the moderate level of stress, \( g_m \) measured at 380 \( \mu \text{mol} \text{ mol}^{-1} \) [\( \text{CO}_2 \)] was unchanged compared with unstressed leaves, while \( g_s \) was reduced by about 80% and \( V_{\text{Cmax}} \) was reduced by about 25% (Table 2). Under the more severe stress, \( g_m \) was reduced by about 50%, with larger reductions in \( g_s \) and \( V_{\text{Cmax}} \). The three stress levels also differed significantly in LWP. \( C_s \) values averaged about 0.69 to 0.78 of \( C_i \) at the different stress levels (Table 2).

The \( C_i \) range of 150 to 400 \( \mu \text{mol} \text{ mol}^{-1} \) did not significantly affect \( g_m \) either for unstressed or severely stressed leaves, based on paired \( t \)-tests for measurements made at each [\( \text{CO}_2 \)] level for each leaf (Table 3). For unstressed leaves, \( g_m \) varied much less from leaf to leaf than did \( g_s \). (see standard errors in Table 2). There was no significant correlation (\( r^2 = 0.14 \), \( n = 7 \) leaves) between \( g_m \) and \( g_s \) in unstressed leaves (not shown).

**Figure 3.** Net CO2 assimilation rate (\( A \)) in relation to substomatal \([\text{CO}_2]\) (\( C_i \)) for Essex soybean leaves at three levels of stress, defined by leaf water potential (LWP). There were six or seven replicate leaves at each level of stress.

Table 2. Mean (±SE) values of leaf water potential (LWP), stomatal conductance (\( g_s \)) to water vapour measured at 380 \( \mu \text{mol} \text{ mol}^{-1} \) [\( \text{CO}_2 \)], maximum rate of carboxylation of Rubisco (\( V_{\text{Cmax}} \)), internal conductance to \( \text{CO}_2 \) (\( g_m \)) and the [\( \text{CO}_2 \)] at the site of carboxylation (\( C_i \)) for soybean leaves at three levels of water stress

<table>
<thead>
<tr>
<th>LWP (MPa)</th>
<th>( g_s ) (mol m(^{-2}) s(^{-1}))</th>
<th>( V_{\text{Cmax}} ) (( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1}))</th>
<th>( g_m ) (mol m(^{-2}) s(^{-1}))</th>
<th>( C_i ) (( \mu \text{mol} \text{ mol}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.74 ± 0.02a</td>
<td>0.690 ± 0.094a</td>
<td>160 ± 10a</td>
<td>0.27 ± 0.01a</td>
<td>200 ± 15a</td>
</tr>
<tr>
<td>−1.41 ± 0.05b</td>
<td>0.120 ± 0.014b</td>
<td>120 ± 8b</td>
<td>0.30 ± 0.02a</td>
<td>148 ± 10b</td>
</tr>
<tr>
<td>−1.86 ± 0.08c</td>
<td>0.048 ± 0.007c</td>
<td>45 ± 12c</td>
<td>0.12 ± 0.02b</td>
<td>143 ± 13b</td>
</tr>
</tbody>
</table>

Each value is a mean for 6 or 7 leaves from different plants. Within a column, values followed by different letters were significantly different at \( P = 0.05 \) by analysis of variance.
DISCUSSION

The mean value of $g_{m}$ at $25^\circ C$ estimated from the O$_2$ sensitivity of photosynthesis in unstressed and moderately stressed Essex soybean leaves was 0.29 mol m$^{-2}$ s$^{-1}$, which compares with an overall mean value of approximately 0.30 (mean values from 0.20 to 0.40 on different days) at the same temperature estimated from fig. 7 in Bernacchi et al. (2005) obtained using fluorescence combined with CO$_2$ exchange, and 0.32 reported by Gillon & Yakir (2000) using O$_2$ isotope discrimination. Thus, the O$_2$ sensitivity method seems to produce reliable estimates of $g_{m}$. One limitation of the method is that [CO$_2$] must remain limiting to net CO$_2$ fixation, which may not be the case at very low temperatures or at very high [CO$_2$] (Sage & Kubien 2007). An important procedural note is that when using absolute infrared analysers to measure CO$_2$ and H$_2$O exchange rates, as do many commercially available photosynthesis systems, the shift in sensitivity of the analysers because of background [O$_2$] needs to be accounted for (Bunce 2002), as the LI-6400 software does.

There was no correlation between $g_{m}$ and $g_{s}$ in unstressed soybean leaves, as Warren (2008a) also found in three species when manipulating $g_{s}$ by changing the leaf to air water vapour pressure difference (D). These results indicate that $g_{m}$ does not directly scale with $g_{s}$. As noted by Warren (2008a), the lack of correlation between $g_{m}$ and $g_{s}$ also indicates that $g_{m}$ was insensitive to the changes in $C_{i}$ resulting from the different $g_{s}$. In the case of soybean, $C_{i}$ at 380 $\mu$mol mol$^{-1}$, external [CO$_2$] varied by about 40 $\mu$mol mol$^{-1}$ from leaf to leaf because of the range of $g_{s}$, which is similar to the $C_{i}$ range reported by Warren (2008a). In soybean, there was no significant change in $g_{m}$ even over a 250 $\mu$mol mol$^{-1}$ range of $C_{i}$ values, both for unstressed leaves and for severely stressed leaves. Loreto et al. (1992) and Bunce (2008) also found no change in $g_{m}$ with $C_{i}$, whereas a significant decrease at high $C_{i}$ has been reported in some species (Centritto et al. 2003; During 2003; Flexas et al. 2007). However, in many of these cases, changes in $g_{m}$ over the range of $C_{i}$ studied here (150 to 400 $\mu$mol mol$^{-1}$) were relatively small, and it is possible that higher $C_{i}$ values would have resulted in lower $g_{m}$ in soybean.

It is clear that soil water deficits can substantially reduce $g_{m}$ in soybean, as also reported in several other species (reviewed in Warren 2008b). In soybean, $g_{m}$ was much less sensitive than $g_{s}$ to water stress, with no change in $g_{m}$ observed at a stress level, which reduced $g_{s}$ by about 80%. However, further reductions in LWP and $g_{s}$ were accompanied by a substantial reduction in $g_{m}$ in soybean. Similar to these results in soybean, Warren (2008a,fig. 5) also found no reduction in $g_{m}$ with mild soil water stress, which decreased $g_{s}$ by about 60% in tomato, but a reduction in $g_{m}$ with more severe stress. In some species, all changes in $g_{s}$ during drought were accompanied by changes in $g_{m}$ (Galmes et al. 2007; Warren 2008a). Reasons for diverse relationships between $g_{m}$ and $g_{s}$ during water stress are unknown, but could be methodological, or related to variation among species in factors contributing to $g_{m}$ (e.g. anatomical versus various biochemical factors).

In the case of soybean, the relative decrease in $g_{m}$ was more similar to the decrease in the photosynthetic parameter $V_{Cmax}$ than to the decrease in $g_{s}$. Two factors often cast doubt on apparent reductions in $V_{Cmax}$ during water stress, errors in $C_{i}$ caused by overestimating $g_{s}$, as it approaches the value of cuticular conductance and errors in $C_{i}$ caused by patchy stomatal closure during stress. However, in the present case, both these potential errors appear to have been minor, because it was observed that after switching leaves from high to low external [CO$_2$], the same $A$ versus $C_{i}$ curve was defined by data before and after $g_{s}$ more than doubled in response to low $C_{i}$. If either patchy closure had occurred or cuticular conductance was significant relative to $g_{s}$, then stomatal opening at low $C_{i}$ would have caused an upward shift in the A versus $C_{i}$ curve.

Drought is one of the most important environmental factors reducing the yield of crops. It reduces yield partly by reducing the efficiency by which intercepted light is converted into plant material through photosynthesis. The inhibition of photosynthesis during drought is highly correlated with reduced stomatal conductance. There has been a long and still unresolved debate about the existence and the importance of factors other than stomatal closure in limiting photosynthesis during drought. Efforts to improve photosynthesis during drought should be based on knowledge of what physiological processes actually limit photosynthesis.

From the earliest gas exchange measurements of leaves during drought (e.g. Brix 1962), it was evident that progressive drought generally causes approximately parallel reductions in $g_{s}$ and $A$. Rather than proving stomatal control of photosynthesis as firstly assumed, however, a truly parallel response would indicate a constant value of substomatal carbon dioxide concentration ($C_{i}$). Hence, drought often reduces $A$ at nearly constant $C_{i}$, or at least $A$ at any given $C_{i}$ value. This seemed clear evidence for non-stomatal inhibition of photosynthesis (Farquhar & Sharkey 1982).

This analysis was upset by the realization that the model that calculates $C_{i}$ from $A$ and $g_{s}$ may not be valid during drought, because it assumes uniform $C_{i}$ across the leaf surface. If a substantial fraction of the reduction in stomatal conductance occurs by complete closure of stomata in patches, then an apparently constant $C_{i}$ can be an artefact of the model (Bunce 1988; Buckley, Farquhar & Mott 1997). Fluorescence measurements indicated a reduction in $C_{i}$ during drought despite a constant calculated value of $C_{i}$ (e.g. Downton, Loveys & Grant 1988), thus pointing towards patchy stomatal closure and stomatal control of photosynthesis. However, Osmond et al. (1999) found fluorescence signals suggesting low $C_{i}$ in stressed plants, while observations of guard cells did not indicate patchy closure, thus raising questions about the interpretation of fluorescence signals as indicating patchy closure in stressed plants. Nevertheless, combined fluorescence and gas exchange measurements during drought on a variety of species led to the generalization that mild and moderate water stress reduced photosynthesis only by closing stomata, but severe
stress resulted in non-stomatal inhibition (reviewed in Flexas et al. 2004). This interpretation needs to be revisited because low carbon dioxide concentrations at the site of carboxylation during drought could potentially occur without stomatal closure, by a decrease in gs. A low Cc can neither be taken as evidence of patchy stomatal closure and stomatal limitation of photosynthesis, nor can the ability to overcome the inhibition by very high carbon dioxide levels.

In soybean, because gs was much less reduced by stress than was gsw, Cc values remained 70 to 80% of Ci even under water stress, but other species may differ in this regard and have low Cc during stress. The method of estimating gsw from the oxygen response of photosynthesis may provide estimates of gm and Cc not subject to the uncertainties of other methods, and allow clearer separation of stomatal and non-stomatal effects of water stress on photosynthesis.

REFERENCES


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