Lateral gas diffusion inside leaves

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Abstract

Diffusion of CO₂ inside leaves is generally regarded to be from the substomatal cavities to the assimilating tissues, i.e. in the vertical direction of the leaf blades. However, lateral gas diffusion within intercellular air spaces may be much more effective than hitherto considered. In a previous work it was demonstrated that, when ‘clamp-on’ leaf chambers are used, leaf internal ‘CO₂ leakage’ beyond the leaf chamber gaskets may seriously affect gas exchange measurement. This effect has been used in the present paper to quantify gas conductance (g_leaf,l, mmol m⁻² s⁻¹) in the lateral directions within leaves and significant differences between homo- and heterobaric leaves were observed. For the homobaric leaves, lateral gas conductance measured over a distance of 6 or 8 mm (the widths of the chamber gaskets) was 2–20% of vertical conductance taken from published data measured over much smaller distances of 108–280 µm (the thickness of the leaves). The specific internal gas diffusion properties of the leaves have been characterized by gas conductivities (g_leaf,l, µmol m⁻¹ s⁻¹). Gas conductivities in the lateral directions of heterobaric leaves were found to be small but not zero. In homobaric leaves, they were between 67 and 209 µmol m⁻¹ s⁻¹ and thus even larger than those in the vertical direction of the leaf blades (between 15 and 78 µmol m⁻¹ s⁻¹). The potential implications for experimentalists performing gas exchange measurements are discussed.

Introduction

Photosynthetic assimilation of CO₂ in the light creates a gradient in CO₂ concentration between the air and the inside of leaves which forces diffusive influx of CO₂. Once inside a leaf, CO₂ moves through air-filled intercellular spaces (ias) as well as liquid (liq) phases of cell walls or matrices. All these provide different gas conductances (g_ias and g_liq, respectively), the combination of which is regarded as the CO₂ transfer conductance (g_w) (Evans and von Caemmerer, 1996). Other terms synonymous with g_w are internal conductance (g_i) or estimated internal conductance (g_est) (Lloyd et al., 1992) as well as mesophyll conductance (g_m) (Loreto et al., 1992; Harley et al., 1992; Parkhurst, 1994). Besides the question of terminology, there is still discussion on whether g_ias or g_liq is the major limiting factor for assimilation (Parkhurst, 1994; Evans and von Caemmerer, 1996; Laisk and Loreto, 1996; Aalto et al., 1999; Evans, 1999; Hanba et al., 1999; Terashima et al., 2001). While g_ias is dominated by diffusion processes in the air, g_liq may be controlled by protein facilitated processes (Bernacchi et al., 2002; Uehlein et al., 2003).

The processes involved in gas movement within leaves have been regarded almost exclusively in the vertical (anticlinal) direction perpendicular to a leaf blade. This might be valid for heterobaric leaves where bundle-sheath...
extensions form narrow compartments within the mesophyll and provide physical barriers to lateral gas diffusion (Neger, 1918). Homobraric leaves, however, lack bundle-sheath extensions and continuous intercellular air space systems may be permeable in both the vertical and the lateral (paradermal) directions. When stomatal closure is unevenly distributed across the leaf surface in heterobraric leaves, mesophyll compartmentation can result in patches of different intercellular CO2 concentrations (ci), while such patchiness is unlikely to occur in homobraric leaves due to lateral gas diffusion (Terashima, 1992). One approach to estimate lateral gas diffusion and to calculate glat is the use of three-dimensional models because CO2 not only spreads to the place of CO2 fixation but spreads in all directions (Parkhurst, 1994). Published studies on lateral gas diffusion within leaves have, up to now, focused on gas transport between neighbouring stomata, i.e. fairly small distances (Terashima, 1992; Parkhurst, 1994). However, lateral gas diffusion may be effective over much larger distances in homobraric leaves and be responsible for artefacts in measured respiration rates (Jahnke and Krewitt, 2002). These authors concluded that this may be a general problem of gas exchange measurements when performed on homobraric leaves with ‘clamp-on’ leaf chambers enclosing only parts of entire leaves.

The goal of the present work was (i) to evaluate whether gas exchange measurements on leaves may be affected by the size of a clamp-on leaf chamber or the width of chamber gaskets, (ii) to quantify gas conductance inside heterobraric or homobraric leaves as a function of diffusion distance in lateral directions, (iii) to calculate gas conductivity as a specific measure of gas diffusion properties of leaves, and (iv) to compare gas conductances and conductivities between the lateral and vertical directions of leaf blades.

Materials and methods

Plant material

Plants (Glycine max (L.) Merr. cv. Williams, Nicotiana tabacum L. cv. Samsun, Phaseolus vulgaris L. cv. Saxa, Vicia faba L. cv. Hangdown Grüinkernig) were grown from seeds in soil (Einheitserde solution (2 mM KNO3, 4 mM Mg(NO3)2.6H2O, 0.8 mM KH2PO4, 0.5 mM MgSO4.2H2O, 1.1 mM CaSO4.2H2O, 11 μM Fe-EDTA (Fetrilon, BASF), 7.5 μM H3BO3, 1.75 μM MnSO4.H2O, 0.08 μM CuSO4.5H2O, 0.13 μM ZnSO4.7H2O, 0.04 μM H3MoO4, 0.003 μM CoCl2.6H2O) adjusted to pH 5.8. Growing conditions were as described in Jahnke (2001), experimental conditions were 23.5 ± 0.5°C, 60 ± 5% RH and CO2 concentrations of either 355 ± 10 μl l−1 or 2000 ± 20 μl l−1 according to the experimental protocol.

G. max and Ph. vulgaris leaves display heterobraric anatomy (Terashima, 1992; Jahnke, 2001) whereas V. faba (Terashima, 1992) and N. tabacum (Jahnke and Krewitt, 2002) are homobraric. All four plant species are characterized by amphistomatous leaves (Napp-Zinn, 1984). Since most of the species taken from the literature were also amphistomatous, a direct comparison of the data obtained here with the published data was facilitated; Ficus carica and Tilia cordata were the only two species to have hypostomatous leaves in the comparison (Napp-Zinn, 1984).

Gas exchange system and leaf chamber design

Gas exchange measurements were performed with an open gas exchange system. The incoming and outgoing CO2 concentration was measured by a differential infrared gas analyser (IRGA; LI-7000, Li-Cor Inc., Lincoln, NE, USA). The dewpoint temperatures of the gas streams entering the reference or analyser cuvette of the IRGA were adjusted to the same value to avoid any problems of water vapour effect on Δ[CO2] measurement. Details of the gas exchange system have been previously described (Jahnke, 2001).

To test the impact of leaf chamber size, two different ‘clamp-on’ leaf chambers were used in the experiments. For the first experiments, a large single-gasket leaf chamber (LLC) with a circular outline, an inner diameter of 7 cm and gasket width of 8 mm was taken to clamp apical parts of leaves enclosing an average leaf area of approximately 25 cm2 (Jahnke and Krewitt, 2002). Atmospheric CO2 concentration inside the LLC was denoted c,a,i whereas [CO2] in the experimental growth cabinet (i.e. outside the leaf chamber) was denoted c,a,o. For other experiments, a double-gasket leaf chamber (LC) with rectangular outlines and gasket width of 6 mm was used. The inner leaf chamber (LCi) enclosed an area of 6 cm2 (2 × 3 cm) while the area between the inner and the outer gaskets (i.e. the outer leaf chamber LCo; Fig. 1) was 15 cm2. Atmospheric CO2 concentrations inside LCi and LCo are denoted c,a,i and c,a,o, respectively. Gas exchange measurements were performed inside LCi whilst LCo was used to change [CO2] at the outer edge of LCi quickly (Fig. 1). To evaluate lateral gas conductance as a function of lateral diffusion length, the inner gasket (Gi) of the LC was removed to achieve a single-gasket leaf chamber with enough space for a stepwise increase of the gasket width between 6, 14, and 22 mm. In these experiments, c,a,i was changed whereas c,a,o (which here was the CO2 concentration in the experimental growth cabinet) was kept constant.

Gas exchange measurements

To determine gas conductance of the mesophyll in lateral directions in leaf blades, experiments were performed in the dark where only respiration contributed to the exchange of CO2. Before measurement, plants were kept in darkness for approximately 36 h as net CO2 exchange rates (NCERs) were stable after that period of time. NCERs were measured as described by Jahnke (2001) under different CO2 concentrations.
concentrations with the following experimental protocol (see Fig. 2a): (i) the experiments started at low $c_{a,o}$ and $c_{a,i}$ (350 $\mu$L L$^{-1}$); (ii) $c_{a,o}$ was increased to 2000 $\mu$L L$^{-1}$ while $c_{a,i}$ was kept unchanged; (iii) $c_{a,o}$ was also increased to 2000 $\mu$L L$^{-1}$; (iv) $c_{a,i}$ was kept high while $c_{a,o}$ was lowered to 350 $\mu$L L$^{-1}$; (v) and finally, the starting conditions (350 $\mu$L L$^{-1}$ on both sides) were re-established.

The properties of the gas exchange system were fully tested in controls. The statistical analysis was performed by ANOVA. Calculations of NCERs and apparent effects of CO$_2$ ($A_{ECO_2}$) on NCERs due to lateral diffusion of CO$_2$ were performed according to Jahnke and Krewitt (2002; where $A_{ECO_2}$ was named ACE).

**Calculation of lateral gas diffusion**

To calculate lateral gas conductance ($g_{\text{leaf}}$) according to Fick’s first law of diffusion (Parkhurst, 1994), the required parameters were obtained experimentally. The area of intercellular air space potentially open for lateral diffusion, $A_{\text{inl}}$, was calculated as:

$$A_{\text{inl}} = L_{\text{gasket}} \times h_{\text{leaf}} \times \text{porosity}$$  \hspace{1cm} (1)

where $L_{\text{gasket}}$ was the length (circumference) of the centre line of the leaf chamber gasket (LC) covering the leaf; $h_{\text{leaf}}$ was the thickness (height) of the leaf blade; and \text{porosity} was the fraction of the volume of intercellular air space and the corresponding leaf volume. Calculation of $A_{\text{inl}}$ by using $L_{\text{gasket}}$ as defined in equation (1) is a simplification of the real situation. For example, for the circular leaf chamber (LLC) the concentric-cylinder geometry of the gaskets should be considered according to Crank (1975). Taking this into account for calculation of conductance (see below) the resulting correction factor was 1.0035 which means conductance was underestimated here by 0.35% when calculation was based on equation (1). This uncertainty was so much below the variability of different measurements that it was not regarded here. To obtain leaf porosity, 8–10 leaf discs per plant were punched out ($r=1.0$ cm), intercellular air space volumes were determined (Jahnke and Krewitt, 2002) and volumes of the leaf discs were calculated as $h_{\text{leaf}} \times r^2 \times \pi$. To determine leaf and tissue thickness, cross-sections of the leaves were made by hand and measured by a microscope with a micrometer scale. Thicknesses of leaves, palisade and spongy tissues, as well as leaf porosities are presented in Table 1.

Diffusive fluxes of CO$_2$ in the lateral directions of the leaf blades ($J_{CO_2,l}$; mmol CO$_2$ m$^{-2}$ s$^{-1}$) were calculated according to:

$$J_{CO_2,l} = (NCER_{\text{ref}} - NCER_l) \times \frac{A_{\text{inl}}}{A_{\text{ref}}}$$  \hspace{1cm} (2)

where $NCER_{\text{ref}}$ was the measured net CO$_2$ exchange rate when [CO$_2$] was identical on both sides of the chamber gasket (i.e. $c_{a,o}=c_{a,i}$) and $NCER_l$ was obtained when there was a difference in external [CO$_2$] between the two sides of the leaf chamber gaskets (i.e. $c_{a,i} > c_{a,o}$ or $c_{a,i} < c_{a,o}$). $A_{\text{inl}}$ was the projected leaf area clamped by the leaf chamber. Finally, lateral gas conductance ($g_{\text{leaf,l}}$; mmol CO$_2$ m$^{-2}$ s$^{-1}$) was calculated as:

$$g_{\text{leaf,l}} = \frac{J_{CO_2,l}}{A_{\Delta c_a}}$$  \hspace{1cm} (3)

with $A_{\Delta c_a} = c_{a,i} - c_{a,o}$.

The calculation of gas conductance is analogous to Ohm’s law of electricity ($I=V/R$) where $I$ is the current, $V$ is the voltage and $R$ is the electrical conductance (Parkhurst, 1994). But for comparison of properties of different systems conductance as such is not very helpful. In electricity, the conductivity $s$ of a conductor was introduced and is defined as $s=I/A$ where $A$ is the cross-sectional area of the conductor (Gettys, 1989). Gas conductance ($g$; mmol CO$_2$ m$^{-2}$ s$^{-1}$) already refers to the diffusion area $A$ (cf. equations 2 and 3) and was taken, in analogy to electricity, to calculate gas conductivity of leaves ($g$; mmol CO$_2$ m$^{-2}$ s$^{-1}$). In the experiments presented here, the path length over which gas diffusion was measured was defined by the width of the chamber gaskets ($w_{\text{gasket}}$; see Fig. 3), and lateral gas conductivity ($g_{\text{leaf,l}}$) of the intercellular air space was calculated according to the equation:

$$g_{\text{leaf,l}} = g_{\text{leaf,l}} \times w_{\text{gasket}}$$  \hspace{1cm} (4)

Conductance can also be expressed as $g=D/\Delta x$ where $D$ describes the diffusivity (diffusion coefficient) and $\Delta x$ the diffusion distance (Nobel, 1991). Multiplication of gas conductance by diffusion distance (equation 4) results in diffusivity which is identical with conductivity. In air, gas diffusivity is well characterized and, as long as the size of the pores does not hamper gas movement, maximum conductivity of an ideal open-porous medium is simply defined by porosity multiplied by the maximum diffusivity in free air (for CO$_2$: $1.51 \times 10^{-5}$ m$^2$s$^{-1}$ or $694$ mmol m$^{-2}$ s$^{-1}$ at 101.3 kPa and 20 °C; Nobel, 1991).

Theoretically, conductivity should not be dependent on the path length of diffusion over which conductance is measured. This was tested by calculating conductivities for the experiments in which the widths of chamber gaskets were changed gradually between 6, 14,
and 22 mm. Published data on gas conductance of leaves almost exclusively deal with gas transport in the vertical direction of a leaf blade. To compare gas conductivities in lateral directions (as investigated here) with those in the vertical direction, published values of vertical gas conductance were taken to calculate vertical conductivities according to equation (4) with the leaf thickness of the particular species taken from the literature.

**Results**

To calculate the potential internal lateral diffusion areas of the plant species investigated, different biometric leaf parameters were collected. Leaf thickness, thickness of spongy and palisade parenchyma, as well as leaf porosity, differed between the heterobaric and homobaric leaves (Table 1). The homobaric leaves were thicker and had significantly higher porosities (53% in broad bean, 38% in tobacco) when compared with the heterobaric ones.

To quantify lateral gas diffusion, apparent rates of respiration were measured in the dark by using a double-gasket leaf chamber (Fig. 1). By changing the CO2 concentrations on both sides of the inner chamber gasket (c\textsubscript{a,i} and c\textsubscript{a,o}; Fig. 2a) potential changes in NCER were evaluated. In heterobaric leaves of G. max, no statistically significant effects of the treatments were observed (Fig. 2b) but, in homobaric V. faba leaves, substantial changes in apparent NCER became obvious (Fig. 2c). Lateral gas conductance of individual leaves (g\textsubscript{leaf,l}) was calculated on the basis of changes in apparent NCER (equations 2 and 3).

Heterobaric leaves of bean and soybean showed negligible lateral gas conductance (g\textsubscript{leaf,l}) whereas, for homobaric leaves of broad bean and tobacco, the values were substantially larger (Table 2). There was a positive relationship for homobaric leaves between biometric leaf parameters and calculated lateral gas conductance: the highest conductances were obtained for broad bean, having thicker leaves and higher leaf porosity than tobacco (Tables 1, 2).

Lateral gas conductance in the heterobaric leaves of Ph. vulgaris and G. max was found to be very small, while

### Table 1. Anatomical leaf parameters of the investigated plant species

<table>
<thead>
<tr>
<th>Leaf anatomy/plant species</th>
<th>n</th>
<th>Leaf (µm)</th>
<th>Palisade tissue (µm)</th>
<th>Spongy tissue (µm)</th>
<th>Leaf porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homobaric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vicia faba</td>
<td>88</td>
<td>479±6 a</td>
<td>184±4 a</td>
<td>255±4 a</td>
<td>53±2 a</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>90</td>
<td>373±5 b</td>
<td>163±3 b</td>
<td>177±3 b</td>
<td>38±1 b</td>
</tr>
<tr>
<td>Heterobaric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>88</td>
<td>229±3 c</td>
<td>95±2 c</td>
<td>99±1 c</td>
<td>32±1 c</td>
</tr>
<tr>
<td>Glycine max</td>
<td>90</td>
<td>188±2 d</td>
<td>84±1 d</td>
<td>81±1 d</td>
<td>32±1 c</td>
</tr>
</tbody>
</table>

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Fig. 3. Schematic drawing of diffusion pathways inside a homobaric leaf when part of the leaf was enclosed in a clamp-on leaf chamber. (a) A cross-section through the double-gasket leaf chamber is drawn with a leaf thickness in due proportion to the gaskets which were 6 mm in widths. (b) Part of (a) is schematically enlarged together with potential diffusion pathways inside the leaf mesophyll; leaf dimensions are drawn out of scale when compared with those of the gaskets. The solid-lined arrow points to the minimum diffusion distance (w\textsubscript{gasket}) which was used to calculate gas conductivities. The ‘true’ diffusion lengths are denoted by dotted-lined arrows and may have been even longer due to tortuosity of the mesophyll (see Discussion). c\textsubscript{a,i}, c\textsubscript{a,o}, atmospheric CO2 concentration in the inner and outer leaf chamber; c\textsubscript{i,i}, c\textsubscript{i,o}, leaf internal CO2 concentration at the inner and outer leaf chamber; J\textsubscript{CO2,i}, lateral diffusional CO2 flux; LI, chamber lid; w\textsubscript{gasket}, gasket width. For the other abbreviations see legend of Fig. 1.
The values of lateral conductivity ($g_{leaf,l}^*$) experimentally obtained from the plant species investigated were clearly smaller (Fig. 5; open symbols) than maximum conductivities in free air (dashed line). Diffusion pathways in the intercellular air spaces of leaves are obstructed by the arrangement of cells inside the mesophyll, and tortuosity ($t$) has thus to be considered (Parkhurst, 1994). Terashima et al. (1996) proposed a tortuosity factor of 1.5 for spongy tissues which was used here to calculate exemplary conductivities corrected for tortuosity (Fig. 5; closed symbols). Gas conductivity of *V. faba* and *N. tabacum* leaves then reached 80% and 42% of the calculated maximum conductivity whereas, when tortuosity was not taken into account (open symbols), the respective values were 52% and 28%.

Lateral gas conductance or conductivity obtained for leaves of a given plant species was almost independent of the leaf chamber type refers to either the large (LLC; 7 cm in diameter) or the small-sized leaf chamber (LCi; 2 cm in diameter) (Table 3). When conductivities as a measure of specific leaf properties were calculated, lateral conductivities ($g_{leaf,l}$) of the homobaric leaves investigated ranged between 67 and 209 μmol m$^{-2}$ s$^{-1}$ as recalculated from published data (Table 3). The effect of diffusion path length on lateral gas conductance was investigated in more detail by increasing the width of the chamber gasket between 6, 14, and 22 mm. This caused obvious changes in gas conductance between 28, 11, and 5.6 mmol m$^{-2}$ s$^{-1}$ (Fig. 4a), whereas gas conductivity was not substantially affected, showing values of 170, 150, and 130 μmol m$^{-2}$ s$^{-1}$, respectively (Fig. 4b).

### Table 2. Lateral gas conductance ($g_{leaf,l}$) and conductivity ($g_{leaf,l}$) inside leaves

<table>
<thead>
<tr>
<th>Leaf anatomy/plant species</th>
<th>Leaf chamber</th>
<th>$g_{leaf,l}$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>$g_{leaf,l}^*$ (μmol m$^{-1}$ s$^{-1}$)</th>
<th>AE$_{CO_2}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Min</td>
</tr>
<tr>
<td>Homobaric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vicia faba</em></td>
<td>LLC</td>
<td>13</td>
<td>26.1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>6</td>
<td>27.8</td>
<td>6.5</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>LLC</td>
<td>14</td>
<td>7.8</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>6</td>
<td>13.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Heterobaric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phaseolus vulgaris</em></td>
<td>LLC</td>
<td>13</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>6</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>LLC</td>
<td>9</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>6</td>
<td>0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* When conductances or conductivities were very low (i.e. around zero), calculation sometimes resulted in negative values which was within the limits of accuracy of the measurements.

### Table 3. Vertical gas conductances ($g_{leaf,v}$) of leaves

The data were collected from the literature and the corresponding vertical gas conductivities ($g_{leaf,v}$) were calculated here by using leaf thickness(vertical diffusion path length) for the particular species found in the literature.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>$g_{leaf,v}$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>Method used</th>
<th>Leaf thickness (μm)</th>
<th>$g_{leaf,v}^*$ (μmol m$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ficus carica</em> L.*</td>
<td>174</td>
<td>CO$_2$ exchange, fluorescence$^a$</td>
<td>262$^a$</td>
<td>46</td>
</tr>
<tr>
<td><em>Gossypium herbaceum</em> L.*</td>
<td>222</td>
<td>Vertical diffusion of N$_2$O$^b$</td>
<td>130$^b$</td>
<td>29</td>
</tr>
<tr>
<td><em>Helianthus annuus</em> L.*</td>
<td>249</td>
<td>CO$_2$ exchange, fluorescence$^c$</td>
<td>280$^c$</td>
<td>70</td>
</tr>
<tr>
<td><em>Tilia cordata</em> Mill.*</td>
<td>141</td>
<td>CO$_2$ exchange, fluorescence$^c$</td>
<td>108$^c$</td>
<td>15</td>
</tr>
<tr>
<td><em>Vigna unguiculata</em> (L.<em>) Walp.</em></td>
<td>176</td>
<td>CO$_2$ exchange, fluorescence$^e$</td>
<td>178$^e$</td>
<td>31</td>
</tr>
<tr>
<td><em>Xanthium strumarium</em> L.*</td>
<td>164</td>
<td>Vertical diffusion of He$^d$</td>
<td>235$^d$</td>
<td>39</td>
</tr>
<tr>
<td><em>Zea mays</em> L.*</td>
<td>154</td>
<td>CO$_2$ exchange, fluorescence$^c$</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>333</td>
<td>Vertical diffusion of CO$_2$</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Vertical diffusion of N$_2$O$^f$</td>
<td>165$^f$</td>
<td>3</td>
</tr>
</tbody>
</table>

$^d$ Farquhar and Raschke (1978).
$^f$ Long et al. (1989).
$^g$ Napp-Zinn (1984).
and (b) the linear function $f(x)=ax+b$. Arithmetical means ± standard error of the mean ($n=6$). Statistical analysis was performed by ANOVA, n.s., non significant; different letters, significant ($P<0.05$) difference between the treatments.

the size and type of the leaf chamber, but the apparent effect of lateral CO$_2$ diffusion on measured NCESS differed largely. When measured with the large-sized leaf chamber, a gradient in CO$_2$ concentration ($\Delta c_a$) of 1650 $\mu$mol mol$^{-1}$ across the chamber gasket caused apparent CO$_2$ effects on NCESS of V. faba and N. tabacum leaves (174% and 59%, respectively; Table 2). Whereas, with the small-sized chamber, the effects were substantially larger (783% and 306%, respectively). For the heterobaric leaves, the effects of $\Delta c_a$ on NCESS were not statistically significant.

Discussion

In the present paper, previous observations that a gradient in CO$_2$ concentration inside the mesophyll of a leaf forces lateral gas movement along intercellular air channels were followed up. Experiments were performed in which leaf respiration was measured in the dark since there is convincing evidence that elevated [CO$_2$] has no instantaneous effect on respiration (Jahnke, 2001; Amthor et al., 2001; Jahnke and Krewitt, 2002; Davey et al., 2004). Any effect on NCESS in the dark due to changes in atmospheric CO$_2$ concentration can thus be interpreted as a consequence of lateral transport of CO$_2$. The magnitude of the effect depends on: intrinsic properties of a leaf (homobaric or not); the very position of where the leaf chamber is clamped on a leaf blade (Jahnke and Krewitt, 2002); and the size of the leaf chamber (Table 2).

Lateral gas conductances and conductivities obtained in the present work can be seen as an approximation of the true values for several reasons. First, the calculated lateral diffusion areas ($A_{\text{leaf,l}}$; equation 1) are the probable maximum values since the effective areas would be smaller when larger veins were located directly under the chamber gaskets. Second, to quantify lateral gas conductance ($g_{\text{leaf,l}}$) accurately it would be best to use the effective differences in leaf internal CO$_2$ concentrations across the chamber gaskets (i.e. $\Delta c_l=c_{l,i}-c_{l,o}$ instead of $\Delta c_a$; cf. Fig. 3b and equation 3). However, since the measured respiration rates in the dark were very low (Penning de Vries, 1975) and differences between $c_a$ and $c_l$ can then be considered as small (Amthor, 1997), calculation of $g_{\text{leaf,l}}$ was simplified by using measured $c_a$ values according to equation (3).

Third, the effective lateral conductivity of the mesophyll was potentially underestimated because measurements included gas movement through the stomata on both sides of the gaskets (Fig. 3b; dotted arrows); i.e. the path length must have been longer than simply the gasket widths ($w_{\text{gasket}}$) used for calculation (equation 4). Fourth, in addition to the previous point, the true path length of diffusion can be considered longer due to tortuosity of the mesophyll; assuming a tortuosity factor of 1.5 (Terashima et al., 1996), the conductivities in Table 2 would be 50% higher which, for demonstration, is drawn in Fig. 5 (closed symbols).

The anatomy of leaves is a major factor in defining internal gas conductivity. In bifacial leaves, spongy parenchyma is generally thicker than palisade parenchyma (Table 2) and spongy tissue has larger porosity than palisade parenchyma (Terashima, 1992). Lateral diffusion in leaves is thus likely to occur preferentially in the spongy parenchyma whereas vertical diffusion encompasses both spongy and palisade tissue. Since air-filled spaces are larger in spongy than in palisade mesophyll, one might expect the gas conductance to be larger in the lateral direction than in
Gas conductance is hyperbolically dependent on the distance of gas diffusion according to Fick’s first law (Fig. 4a). In order to facilitate a direct comparison of tissue-specific properties, gas conductivity was derived from measured gas conductance. In analogy to electrical conductivity describing the general property of a conductor, independent of its size or form (Gettys, 1989), gas conductivity is independent of the path length of diffusion. But when measured in homobaric V. faba leaves, gas conductivity showed a small decrease with increasing path length (Fig. 4b). This can be explained by the fact that, in a given leaf, intercellular air space is not simply a homogeneous system but may vary throughout a leaf blade. In Fig. 4b, lateral conductivity as calculated by linear regression amounted to 185.3 μmol m⁻¹ s⁻¹ and was very close to the mean of all conductivity data (195 μmol m⁻¹ s⁻¹) obtained from V. faba leaves (Table 2). In general, the measured gas conductivities in the lateral directions of homobaric leaves were markedly higher than those in the vertical direction recalculated from the literature (Tables 2, 3).

The results show that lateral gas diffusion inside leaves can vary substantially between different species. Lateral gas conductivity of heterobaric leaves was very low due to the compartmentation of the mesophyll (Neger, 1918). For homobaric leaves, the observed data of lateral gas diffusion were highly variable. This can be explained by the fact that (effective) lateral diffusion areas within leaf blades, defined by shape and size of intercellular air spaces, may vary between individual leaves or experiments. Large veins completely prevent gas movement in lateral directions; minor veins may be more or less prominent and can also obstruct gas diffusion to varying degrees. In leaves where veins of different orders are quite differently shaped as in N. tabacum, the variability of the experimental results was particularly large because the mere position of where the leaf chamber was clamped affected the measurements of NCERs (Jahnke and Krewitt, 2002). It obviously also caused the large differences between minimum and maximum values of the derived parameters presented in Table 2 (g_{leafh}, g_{leafl}^* and AECO₂). However, even in heterobaric leaves, sometimes small but considerable lateral gas fluxes were measured (Table 2). There must be a broad variability of interconnectivity inside intercellular gas spaces of leaves which can be modified by plant internal constraints (e.g. genetics or stage of development) as well as external ones (e.g. exposure to light or temperature). The present results indicate that, at least inside homobaric leaves, gas conductivity can be larger in the lateral than in the vertical direction and leaf internal gas transport can be higher than usually considered. All this may have implications for experimentalists. Whenever there is a difference in CO₂ concentration between the inner and outer section of a leaf chamber, leaf internal gas fluxes may affect measurements. This is a point particularly when clamp-on leaf chambers are small in size. The chamber size defines the ratio between length (circumference) of the leaf chamber gasket and the enclosed leaf area; the larger the ratio the larger the potential effect on measured NCER eventually causing erroneous results. For reliable gas exchange measurements, small leaf chambers are therefore not the appropriate tools. Whether lateral gas movement inside leaves could be relevant for the physiology of leaves has still to be evaluated.

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