LIMITATIONS AND BENEFITS OF OXYGEN DIFFUSION CONTROL IN LEGUME NODULES

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ABSTRACT
Evidence is presented that the diffusion of oxygen into the bacteroid zone of legume nodules is regulated by a variable barrier. The implications of this in relation to oxygen limitation of nitrogen fixation and protection of nitrogenase during periods of environmental stress are reviewed.

INTRODUCTION
All aerobic nitrogen fixing organisms have a problem with oxygen. It is required for the production of ATP by oxidative phosphorylation but direct contact with nitrogenase leads to irreversible damage. In legume nodules the solution is to restrict oxygen supply to the bacteroid zone by means of a diffusion barrier. High rates of respiratory oxygen consumption are maintained in the bacteroid zone, with leghaemoglobin providing facilitated diffusion within the infected cells. It is now becoming generally recognized that the resistance of the diffusion barrier can be varied in a controlled manner. The implications of this diffusion control for nitrogen fixation in legumes are the subject of this review.

EVIDENCE FOR A VARIABLE BARRIER
Oxygen moves through air about $10^4$ times faster than through water. Consequently air pathways which traverse the nodule offer a major route for the distribution of oxygen. In relation to these pathways, nodule structure can be divided into four zones (Witty et al., 1986):
   a) the outer cortex through which oxygen can diffuse freely via a network of air pathways,
   b) the inner cortex which appears to have very few air pathways,
   c) the boundary between the inner cortex and the bacteroid zone which had a proliferation of interconnected pathways, and
   d) the bacteroid zone which also has a substantial network of pathways.

Thus, the inner cortex appears to be the major impediment to diffusion. Further evidence for this has been provided by studies in which microelectrodes are gradually advanced into the bacteroid zone. For functional nodules, these show a sharp decrease in oxygen concentration across the inner cortex and very low concentrations in the bacteroid zone (Tjepkema and Yocum, 1974; Witty et al., 1987).

Indirect evidence for variability of the diffusion barrier has come from studies using intact nodulated plants, or detached nodules, in flow-through gas systems. These are summarized in Table 1.

**TABLE 1. Indirect evidence for a variable diffusion barrier**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1) Exposure to C$_2$H$_2$ causes a rapid decline in C$_2$H$_4$ and CO$_2$ production by nodules</td>
<td>Minchin et al., 1983b</td>
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<td>2) This decline is reversible</td>
<td>Minchin et al., 1983a</td>
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<td>3) A 4-fold increase in O$_2$ from 21 to 80% caused only a 36% increase in respiration, but nitrogenase was not damaged</td>
<td>Sheehy et al., 1983</td>
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<td>4) Nitrogenase is damaged if O$_2$ is increased from 21 to 80% in less than 1 minute.</td>
<td>Witty et al., 1984</td>
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To understand the implications of the first three observations it is necessary to consider Fick's first law of gaseous diffusion. For nodules this can be written as:

$$F = \left(\frac{O_e - O_i}{R}\right)$$

where $F = $ oxygen flux into the nodule, $O_e = $ external oxygen concentration, $O_i = $ internal oxygen concentration, and $R = $ diffusion resistance.

Assuming arbitrary units of $F = 10$, $O_e = 20\%$, $O_i = 0\%$ (to which it must approximate if nitrogenase is functional) and $R = 2$, then a decrease in $F$ to 5 (simulating a 50% acetylene-induced decline in respiratory O$_2$ consumption) with $O_e$ and $R$ remaining constant would produce an $O_i$ value of about 10%, resulting in nitrogenase damage. The acetylene-induced decline is reversible, indicating that nitrogenase is not damaged and $O_i$ has stayed close to zero. This can only be achieved by a doubling of $R$. Similarly, an increase in $O_e$ to 80% without a concomitant increase in respiratory oxygen consumption (i.e. $F$ remaining constant) would, if $R$ also remained constant, result in $O_i$ approaching 60%. Since nitrogenase is not damaged (as evidenced by high rates of activity when $O_e$ is