Effect of pO\textsubscript{2} during Growth on the Gaseous Diffusional Properties of Nodules of Cowpea (Vigna unguiculata L. Walp.)

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ABSTRACT

Adaptations of nodules of cowpea (Vigna unguiculata L. Walp. cv Vita 3: Bradyrhizobium CB 756) to growth in pO\textsubscript{2} ranging from 1 to 80% O\textsubscript{2} (volume/volume) involved both readily reversible mechanisms of adjustment and more stable alterations which together resulted in nodules with widely ranging resistance to diffusion of gases. Those grown in subambient pO\textsubscript{2} (1–5% O\textsubscript{2}) were altered such that rapid diffusional adjustment was unable to prevent irreversible loss of nitrogenase on their transfer to higher levels of O\textsubscript{2}. Those cultured in 80% had adapted to over-supply of O\textsubscript{2} such that their transfer to lower levels of O\textsubscript{2} limited both nitrogenase and respiratory CO\textsubscript{2} release. There was also some evidence for ‘protective respiration.’ Measurement of diffusional properties based on gas exchange kinetics indicated that gaseous permeability values for nodules from 5 to 40% O\textsubscript{2} were relatively constant around 20 x 10\textsuperscript{-3} millimeters per second, while those for nodules from 1% O\textsubscript{2} were as high as 67.7 x 10\textsuperscript{-3} millimeter per second and from 80% as low as 6.8 x 10\textsuperscript{-3} millimeters per second. Estimates of the thickness of the diffusion barrier ranged from 75 micrometers for nodules from 1% O\textsubscript{2} to 71.9 micrometers in those from 80% O\textsubscript{2}.

Despite suggestions that legume nodule functioning in normal air may be limited by O\textsubscript{2} supply (see Dakora and Atkins [4] for review), fully symbiotic cowpea plants showed a broad plateau of growth and relatively constant rates of N\textsubscript{2} fixation at pO\textsubscript{2} ranging from 5 to 40 or 60% (5). At sub- or supra-ambient concentrations outside this range, however, there was substantial inhibition of fixation and consequently plant growth was limited (5). A similar lack of response to altered O\textsubscript{2} level around the roots of a number of legume (9) and nonlegume (11, 12) symbioses have been noted. Mechanisms which result in the plant being able to maximize N\textsubscript{2} fixation over such a broad range of pO\textsubscript{2} have not been identified. However, recent studies (8, 10, 13, 15–17), which have demonstrated the operation of variable barrier to the movement of gases in legume nodules, indicate that adaptation may involve altered diffusional properties.

In the present study, cowpea plants, cultured with their nodulated roots maintained in a range of sub- and supra-ambient pO\textsubscript{2}, were used to more closely examine the nature of the adaptation of nodules to altered O\textsubscript{2} level. Measurements involved assays of nitrogenase and CO\textsubscript{2} evolution following transfer of intact root systems to O\textsubscript{2} levels different from those used for their growth and estimates of the permeability of nodules derived from kinetics of gas exchange.

MATERIALS AND METHODS

Plant Material and Analysis

Cowpea (Vigna unguiculata L. Walp. cv Vita 3) plants, inoculated with Bradyrhizobium strain CB 756, were grown in liquid culture as described previously (5) with their whole nodulated root system, or crown root nodulated zone only, maintained in atmospheres containing a range of pO\textsubscript{2} (1–80%, v/v) in N\textsubscript{2}. Plants were maintained under these conditions for varying periods up to 56 d after planting or had the composition of the gas streams serving culture vessels changed with respect to O\textsubscript{2}. Experimental details relating to time of culture under a particular pO\textsubscript{2}, transfer of plants to altered pO\textsubscript{2}, and the timing of assays during culture are indicated with the results.

Plants were harvested during culture to determine the diameter of nodules and for measurement of total plant N by Kjeldahl digestion.

Nitrogenase Assay

Nitrogenase (EC 1.7.99.2) activity was measured as ethylene production in a flowing gas stream containing 10% acetylene and a range of pO\textsubscript{2} as described previously (5).

Measurement of CO\textsubscript{2} Evolution

CO\textsubscript{2} produced by enclosed whole nodulated root systems or nodulated crown root zones was determined as described earlier (5) by measuring the CO\textsubscript{2} content of the gas stream serving cultures before and after passage through the enclosing vessels. In experiments where the pO\textsubscript{2} of the gas stream was altered over 20-min periods, CO\textsubscript{2} evolution was measured at the same time as ethylene formation in a stream containing 10% (v/v) acetylene.

Determinant of Gaseous Diffusional Properties

The diffusional properties of nodules on plants cultured with their root crown nodulation zones in different pO\textsubscript{2} were

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studied experimentally with a model based on the nonsteady-state, 'lag phase' method of ethylene production measurement developed by Davis (7). This model is comparable, in the description of its physical parameters, to that of Weisz and Sinclair (14). The small culture vessels, which enclosed the growing nodulated root crown, served as the gas exchange cuvettes. Their internal volume was 10 mL so that, with a gas flow rate of 300 mL·min⁻¹, the chamber had a time constant of 2 s. The pO₂ of the gas stream passing through the chambers throughout the growth of plants was maintained during assays of acetylene reduction. The assay was initiated by changing the gas stream to one containing 10% (v/v) acetylene and samples (1 mL) taken from the effluent stream at recorded time intervals of approximately 4 s for up to 160 s following transfer. Ethylene content was measured in these samples to establish the pattern of ethylene exchange by nodules on plants cultured for varying periods (44–56 d after planting) with their nodulated crown root in a range of pO₂ (1 to 80%, v/v).

RESULTS AND DISCUSSION

Response to and Adaptation of Plants Grown at Sub- or Supra-ambient O₂ to Changes in pO₂

If the broad plateau of N₂ fixation and plant growth which was found previously for cowpea with O₂ levels from 5 to 60% (5) reflected adaptations involving operation of a variable diffusion barrier, then some evidence for adjustment might be seen in the response of nodulated root systems of these plants to sharp changes in O₂ level. Nodules grown at subambient O₂ should have lowered diffusion resistance and those at high O₂ should have increased diffusion resistance, such that when each is transferred rapidly to higher and lower O₂ concentration, respectively, those from low O₂ would suffer from effects due to O₂ excess, while the reverse should obtain for those from high O₂ culture. The changes in acetylene reduction following transfer of nodulated root systems of cowpea from a range of pO₂ to air indicate that this prediction was largely realized (Table I). Ten min following exposure to air, activity of transferred plants decreased in all cases and remained depressed for up to 24 h. By 3 d, nodules cultured in O₂ levels from 5 to 60% had reestablished rates of acetylene reduction in air similar to those in their original O₂ level at time zero. However, those from 1 or 2.5% O₂ did not establish rates commensurate with their new O₂ supply until 15 d, while those from high O₂ (80%) showed steadily increasing rates of reduction which, by 15 d, were more than 10-fold those at time zero (Table I). These changes in acetylene reduction were reflected in incremental changes in total plant N recorded over the 15-d period of the experiment (Fig. 1). In plants cultured in subambient O₂, a combination of initial inhibition and subsequent stimulation of nitrogenase on transfer to air resulted in N increments being similar to those of control plants maintained throughout at the pO₂ used for culture. Those from 60 and 80% O₂, on the other hand, showed significantly greater increments of N when transferred to air (Fig. 1). In those transferred from 80% O₂, the amount of N fixed over the 15-d period (15 ± 3 mg·plant⁻¹, Fig. 1) was comparable to the activity of plants maintained in normal air for 35 d (19 ± 8 mg·plant⁻¹).

Transfer of 20-d old, air-grown cowpea plants into a range of different O₂ levels also revealed significant adjustments in nitrogenase activity 24 h after altering the O₂ (Table II). At

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**Table I. Nitrogenase Activity of Cowpea Plants Grown in Different pO₂ for 20 d and Then Transferred to Air until 35 d after Planting**

Control plants were not transferred to air but were maintained at the original pO₂ used for their growth. Controls were assayed for nitrogenase activity in the pO₂ used for their culture while those transferred to air were assayed in air. Both types of plants were assayed at the same time.

<table>
<thead>
<tr>
<th>pO₂ during Culture</th>
<th>Time after Transfer to Air (h)</th>
<th>μmol C₂H₄ produced·h⁻¹·plant⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferred</td>
<td>0.8 ± 0.09a</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferred</td>
<td>1.9 ± 0.10</td>
<td>1.3 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferred</td>
<td>3.3 ± 0.44</td>
<td>2.1 ± 0.13</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferred</td>
<td>7.1 ± 0.09</td>
<td>6.6 ± 0.15</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.3 ± 0.62</td>
<td>9.4 ± 1.12</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.5 ± 0.20</td>
<td>4.7 ± 0.26</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.6 ± 0.28</td>
<td>4.8 ± 0.19</td>
</tr>
<tr>
<td>Transferred</td>
<td>5.2 ± 0.11</td>
<td>3.5 ± 0.15</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.5 ± 0.11</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Transferred</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not assayed.  
^ Means ± SE (n = 4).  
^ Assays at time = 0 were at the pO₂ used for growth of plants.
Values are transferred to in plantings in maintained 1. 958 DAKORA c

Figure 1. Changes in total N of cowpea cultured in different pO2 and transferred to air for 15 d. These plants were grown to 20 d after planting in different O2 levels and some were harvested for total N, others were transferred to air for 15 d, while those remaining were maintained in their original pO2 as controls for the 15-d period in air. Values are means ± se (n = 4). DAP = days after planting inoculated seed.

1% O2, air-grown plants showed a sharp decrease, while at other O2 levels, above and below that of air, activity was only slightly reduced even after 48 h. By 72 h, the broad plateau of activity from 2.5 to 60% O2 indicated effective adjustment so that fixation of N2 was optimized over this range. Davey and Simpson (6) have reported similar results for subterranean clover plants grown in air and transferred to subambient pO2. Although respiration and acetylene reduction were initially depressed, by 24 h both had been reestablished to the original rates in air. Criswell et al. (2) also indicated that 24 h was required for soybean nodules to adapt to sharp declines in pO2. Interestingly, after 48 h at 1% O2, nitrogenase activities rose steadily and by 144 h had reached values around 50% of those in air. In contrast, those transferred to 80% O2 showed only a small initial decrease in activity which was not reversed; in fact, activity continued to fall slowly at rates reduced by 80% or more by 144 h (Table II). At 144 h, all plants were transferred back to air and acetylene reduction was assayed again after 10 min. Except those which had been in air throughout, or had been transferred to 10% O2, all showed a significant decline in nitrogenase activity (Table II). This was most marked for plants which had been held in low O2; reductions of 92, 87 and 79% were observed for those transferred from 1, 2.5, and 5% O2, respectively.

These data clearly indicate long-term adaptation of nodules so as to maximize nitrogenase activity either under conditions of over- or undersupply of O2 in the external gas phase. Within the range of 5 to 60%, O2, adaptations were apparently easily altered and effective adjustments could be made within 24 h. It seems reasonable to suppose that mechanisms controlling what has been described as the 'variable diffusion barrier' (16) are major components of this response. However, with severely limiting subambient O2 and levels of O2 above 60%, readjustment was much slower, suggesting that adaptation also involved more extensive, possibly structural, modifications altering the organ's permeability to gases.

The sharp changes in pO2, for example in transferring nodules from 1 to 2.5% O2 to air, or from air to 80% O2, could have resulted in inactivation of nitrogenase with part of the adaptation to change requiring some measure of control.

Table II. Rates of Acetylene Reduction of 20-d-Old, Air-Grown Cowpea Plants Following Transfer to Different pO2 for 6 d

<table>
<thead>
<tr>
<th>O2 level</th>
<th>Time after Transfer to Different pO2 (h)</th>
<th>At 10 min after Transfer Back to Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>10 min after Transfer Back to Air</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>144</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.5 ± 0.03 0.3 ± 0.03 1.9 ± 0.21 3.2 ± 0.37 3.3 ± 0.12</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>2.5</td>
<td>4.4 ± 0.08 4.5 ± 0.18 5.4 ± 0.32 5.6 ± 0.34 5.7 ± 0.53</td>
<td>0.7 ± 0.15</td>
</tr>
<tr>
<td>5</td>
<td>5.8 ± 0.51 6.2 ± 0.07 5.6 ± 0.41 5.1 ± 0.30 5.7 ± 0.25</td>
<td>4.6 ± 0.55</td>
</tr>
<tr>
<td>10</td>
<td>7.1 ± 0.27 6.7 ± 0.14 6.6 ± 0.14 6.0 ± 0.39 5.6 ± 0.25</td>
<td>4.6 ± 0.55</td>
</tr>
<tr>
<td>20</td>
<td>5.3 ± 0.17 7.3 ± 0.58 7.0 ± 0.40 4.9 ± 0.21 4.6 ± 0.14 5.5 ± 0.65</td>
<td>5.4 ± 0.89</td>
</tr>
<tr>
<td>40</td>
<td>7.2 ± 0.24 4.4 ± 0.28 4.0 ± 0.22 3.8 ± 0.15 4.4 ± 0.69</td>
<td>3.8 ± 0.46</td>
</tr>
<tr>
<td>60</td>
<td>6.3 ± 0.11 5.7 ± 0.26 4.6 ± 0.60 4.3 ± 0.85 3.7 ± 0.25</td>
<td>1.6 ± 0.27</td>
</tr>
<tr>
<td>80</td>
<td>5.2 ± 0.36 4.9 ± 0.59 2.2 ± 0.21 2.0 ± 0.29 1.6 ± 0.27</td>
<td>0.8 ± 0.17</td>
</tr>
</tbody>
</table>

a Not assayed. b Mean ± se (n = 6).
of O₂ supply before the level of functional enzyme could be re-established. Sheehy et al. (10) and Witty et al. (15) have reported that short-term increases in external gas phase pO₂, if applied in a gradual stepwise manner, do not result in nitrogenase inactivation but rather allow the nodule to adapt by increasing diffusive resistance. Thus, it was of interest to examine the short term response to gradual changes in pO₂ by nodules already adapted to optimal nitrogenase activity in sub- or supra-ambient O₂. Plants were cultured with their nodulated root crowns in 2.5 or 5% O₂ for 40 d and then exposed to stepwise increases over subsequent 20-min periods, finally reaching 20% O₂. Toward the end of each 20-min period, rates of acetylene reduction and CO₂ evolution were measured. In both cases, nitrogenase activity decreased sharply and progressively with increasing pO₂ (Fig. 2A), while CO₂ evolution was essentially unaffected. This response, interpreted in the simplest way, indicated that the variable diffusion barrier in these low O₂-adapted nodules was not able to adjust to the over-supply of O₂ and that nitrogenase was inactivated. Transferring these plants back to low O₂ for 45 to 60 min did not restore the original rates of activity. This does not preclude the operation of a variable barrier in these low-O₂ grown nodules. More gradual increases in pO₂ might have allowed adjustment and prevented the rapid loss of nitrogenase.

Similarly, plants grown with their nodulated root crown in 80% O₂ and transferred more gradually to lower O₂ levels also showed a sharp and progressive decline in acetylene reduction (Fig. 2B). In this case, however, CO₂ evolution declined progressively as pO₂ was reduced (Fig. 2B) and, unlike those transferred from lower to higher pO₂, restoring the original pO₂ to the rooting atmosphere resulted in almost complete recovery of both nitrogenase activity and CO₂ evolution (data not shown). Thus, the response of acetylene reduction was not due to changes in the level of active nitrogenase enzyme but rather reflected a declining supply of respiratory products (ATP, reductant). Inhibition of CO₂ evolution by stepwise declines in pO₂ could have been due to the fact that the resistance of these 80% O₂-grown nodules was sufficiently high that internal pO₂ was already limiting to respiration. However, in view of the relatively prompt response, for example in going from 80 to 60% O₂, this seems unlikely. Alternatively, a large proportion of the CO₂ evolution in these nodules could have been a consequence of electron transfer through an alternate, ‘low affinity’ terminal oxidase (1). That this might be the case for cowpea nodules grown in supra-ambient O₂ was suggested previously (5) by measurements of CO₂ evolution which indicated relatively inefficient respira-

Figure 2. Relationship between acetylene reduction and CO₂ production by the nodulated root crown of cowpea with stepwise increases in pO₂ from (A) 2.5 or 5% to 20% O₂ using plants cultured with their nodulated root crown in 2.5 or 5% O₂, respectively, for 40 d and (B) from 80% to 1% O₂ using plants cultured with their nodulated root crown in 80% O₂ for 40 d. Values are means ± se (n = 4). Arrows indicate the direction of change of O₂ from one level to the next, which in each case occurred in 20 min. Assays were carried out towards the end of each 20-min period.
tion by those grown at 80% O2. The operation of some sort of 'protective respiration' seems a logical adaptation by nodules cultured in supra-ambient O2, and Bergersen and Turner (1) envisaged such a mechanism as being effectively uncoupled from phosphorylation. Although the data of Figure 2B support the involvement of protective respiration, nitrogenase activity was directly dependent on this respiration, and it seems clear that the process, or some component of it, generated ATP. Whether the operation of such an alternate pathway of electron transfer, not involving ATP synthesis, occurred when the low pO2-grown nodules were transferred to higher O2 level (Fig. 2A), or whether the continued high rate of CO2 evolution at negligible nitrogenase activities was possible through some alternate use for ATP, cannot be ascertained.

**Diffusional Properties of Cowpea Nodules Exposed to Different pO2 during Growth**

Following exposure of nitrogenase-containing nodules to acetylene, there was a lag in time before a steady rate of ethylene evolution was established (see refs. 5, 7, and 14). This lag, which is likely to be a function of the diffusivity of the gas through the nodule, was determined from almost continuous assays of acetylene reduction during the first minute or two following exposure of nodules to the gas. pO2 had a marked effect on the time required for ethylene efflux to reach a maximum rate. The data of Table III, derived from a series of time courses for 3 or 4 replicate plants at each pO2 and determined at 36, 40, 44 and 56 days after planting, indicated that the lag time increased with the level of O2 around the root segment. Although nodules at low O2 were smaller than those in air, there was not a close relationship between size and the lag in ethylene efflux (Table III); those at supra-ambient O2 were also smaller than those in air but showed a considerably greater lag time.

From measured values of the rate of ethylene efflux (expressed as mm3·s−1) with time up to a maximum or steady state value, time constants for nodules at each pO2 were calculated using the exponential function:

\[ F_e = F_{io} \times (1 - e^{-t}) \]

where \( F_e \) is the rate of efflux, \( F_{io} \) is the maximum rate of efflux (mm3·s−1), \( t \) is time (s), and \( r \) is the time constant (s). These values which are indicated in Table III, were used with the model for gaseous exchange of soybean nodules developed by Weisz and Sinclair (14) to estimate gaseous permeability. The same assumptions as those used for soybean were made for cowpea nodules and, as in the model of Weisz and Sinclair (14), the inner cortex of the nodule was envisaged as the major component of diffusive resistance. However, in cowpea nodules cultured in different pO2, the thickness of this tissue component varied widely (3) so that values from 4 to 100 μm were used in the simulation. Similarly, the range of nodule diameters used accommodated the diameters of nodules for cowpea cultivated at different pO2 (Table III).

The average gaseous permeability values for soybean nodules (13.9 × 10−3 mm3·s−1·cm−1) found by Weisz and Sinclair (14) are comparable to those for cowpea nodules grown in air (around 20 × 10−3 mm3·s−1·cm−1, Table III). However, nodules from subambient pO2 were significantly more permeable to gas, while those at increasingly supra-ambient levels were progressively less permeable.

The effect of pO2 on the permeability of nodules to gases is quite consistent with their adaptations to over- or undersupply of O2 demonstrated by the transfer experiments described above. At the extremes of low and high O2, the differences seem to be fairly clear. Nodules from low pO2 are considerably more permeable to gas, and as a consequence even small increases in gas phase O2 levels are toxic. Limitation in nitrogenase activity with even small decreases in external O2

### Table III. Effect of pO2 on Diameter of Nodules, Their Gaseous Permeability, Associated Time Constant for Gas Exchange and Thickness of the Diffusion Barrier

Cowpea plants were grown with the nodulation crown zone exposed to different pO2 from 8 d after planting. Measurements were made at 36, 40, 44, and 56 d after planting and in the pO2 used for growth.

<table>
<thead>
<tr>
<th>pO2</th>
<th>Nodule Diameter</th>
<th>Lag Time</th>
<th>Fw</th>
<th>Time Constant</th>
<th>Gaseous Permeability</th>
<th>Thickness of Diffusion Barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>mm</td>
<td>s</td>
<td>mm3·s−1</td>
<td>s</td>
<td>mm·s−1·10^3</td>
<td>μm</td>
</tr>
<tr>
<td>1</td>
<td>2.16 ± 0.20</td>
<td>20.8 ± 5.4</td>
<td>70.6 ± 19.6</td>
<td>3.4 ± 0.6</td>
<td>67.7 ± 6.0</td>
<td>7.5 ± 1.0</td>
</tr>
<tr>
<td>2.5</td>
<td>3.17 ± 0.10</td>
<td>29.3 ± 4.0</td>
<td>168.7 ± 34.3</td>
<td>6.0 ± 0.6</td>
<td>63.7 ± 6.9</td>
<td>9.3 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>3.17 ± 0.20</td>
<td>52.0 ± 6.6</td>
<td>155.4 ± 28.2</td>
<td>13.9 ± 2.7</td>
<td>30.7 ± 3.3</td>
<td>17.6 ± 1.5</td>
</tr>
<tr>
<td>10</td>
<td>3.53 ± 0.24</td>
<td>69.3 ± 4.5</td>
<td>595.2 ± 46.1</td>
<td>18.6 ± 1.8</td>
<td>19.8 ± 1.3</td>
<td>24.3 ± 0.8</td>
</tr>
<tr>
<td>20</td>
<td>3.95 ± 0.33</td>
<td>87.6 ± 5.6</td>
<td>653.9 ± 163.6</td>
<td>22.6 ± 3.2</td>
<td>20.7 ± 3.2</td>
<td>22.3 ± 1.5</td>
</tr>
<tr>
<td>40</td>
<td>3.15 ± 0.20</td>
<td>96.3 ± 3.0</td>
<td>227.0 ± 36.8</td>
<td>29.1 ± 2.9</td>
<td>17.1 ± 1.4</td>
<td>27.5 ± 0.7</td>
</tr>
<tr>
<td>60</td>
<td>3.60 ± 0.30</td>
<td>101.5 ± 7.6</td>
<td>342.8 ± 59.7</td>
<td>35.0 ± 4.7</td>
<td>13.8 ± 1.6</td>
<td>39.1 ± 1.6</td>
</tr>
<tr>
<td>80</td>
<td>2.36 ± 0.53</td>
<td>104.0 ± 6.1</td>
<td>134.5 ± 32.5</td>
<td>52.8 ± 4.8</td>
<td>6.8 ± 1.5</td>
<td>71.9 ± 2.0</td>
</tr>
</tbody>
</table>

* a Mean ± SE (n = 23–40 nodules at each of four sampling times).

b Determined from time courses of ethylene efflux as the time (s) to reach Fw; Values are means ± SE (n = 3–4 plants at each of four sampling times).

c See text for definition and determination.

d Derived by dividing acetyldec diffusivity in plant tissue (4.5 × 10−4 mm2·s−1; see Weisz and Sinclair [14]) by estimates of gaseous permeability (this table).
by those from 80% is likewise consistent with their low gaseous permeability. However, those in the range over which nitrogenase levels were effectively maximized (5-40% O₂) show remarkably constant values for gaseous permeability (Table III). As discussed, both variable diffusion resistance and more stable modifications of nodule structure appear to be components of this response. It is not possible from these data to assign relative importance to each of these in determining the nodule’s adaptation. However, it will be interesting to compare measurements of the relative sizes of tissue components of nodules, particularly of the thickness of the inner cortex, with estimates for the thickness of the diffusion barrier as provided by this study over a range of pO₂.

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