Plant Physiol. (1994) 106: 263–270

Acclimation of Soybean Nodules to Changes in Temperature[1]

Monika M. Kuzma and David B. Layzell*

Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

This study examines how O_2 status, respiration rate, and nitrogenase activity of soybean (*Glycine max*) nodules acclimate to short-term (<30 min) temperature change from 20 to 15°C or from 20 to 25°C. Acclimation responses were compared between nodules on uninhibited plants and nodules that were severely O_2 limited by exposure to Ar:O_2. In uninhibited nodules the decrease in temperature caused a rapid inhibition of nitrogenase activity followed by partial recovery, whereas in Ar:O_2-inhibited nodules the temperature decrease caused a minor stimulation followed by a gradual decline in nitrogenase activity. In contrast, the temperature increase caused a gradual increase in nitrogenase activity in uninhibited nodules, and an initial inhibition followed by a rapid rise in Ar:O_2-inhibited nodules. In both uninhibited and Ar:O_2-inhibited nodules, temperature had only minor effects on the degree to which nitrogenase activity was limited by O_2 supply, but nodule permeability to O_2 diffusion was greater at 25°C, and less at 15°C, than that measured at 20°C. On the basis of these data, we propose that temperature change alters the nodule's respiratory demand and that the observed changes in nodule permeability occur to maintain control over the infected cell O_2 concentration as the O_2 demand increases at high temperature or decreases at low temperature.

Temperature is an important environmental variable that strongly affects most biochemical processes, including N_2 fixation. At low temperature, nitrogenase activity and nitrogenase-linked respiration decline, whereas at high temperatures, they rise to reach a maximum value between 20 and 35°C, depending on the legume-bacteria symbiosis (Dart and Day, 1971; Pankhurst and Sprent, 1976; Munevar and Wollum, 1981; Layzell et al., 1984; Pankhurst and Layzell, 1984; Ryle et al., 1989; Ofosu-Budu et al., 1992).

Because the nitrogenase enzyme is O_2 labile yet requires oxidative phosphorylation to meet its energy and reductant requirements, changes in nitrogenase activity must be coordinated with the regulation of O_2 diffusion into the nodules. To achieve this, legume nodules control their permeability to O_2 diffusion to maintain an *Oi* in a range that limits their metabolism yet permits oxidative phosphorylation (Hunt and Layzell, 1993). Although nitrogenase activity appears to be O_2 limited even under optimal conditions (Hunt et al., 1989), treatments such as NO_3^- fertilization (Vessey et al., 1988), nodule disturbance (Minchin et al., 1986), nodule detachment (Sung et al., 1991), restriction of phloem sap supply to the nodule (Vessey et al., 1988), exposure of nodules to an Ar:O_2 atmosphere (King and Layzell, 1991; Kuzma et al., 1993), and exposure to 10% acetylene (Minchin et al., 1983) have been shown to make nodules more O_2 limited by decreasing their permeability to O_2 diffusion and thereby decreasing *Oi*.

Few studies have examined the role of O_2 in the regulation of nodule metabolism during changes in temperature, and virtually nothing is known about the dynamics of nodule metabolism and O_2 diffusion during a change in nodule temperature. Low temperature is known to increase O_2 solubility and decrease its diffusion coefficient (Altman and Dittmer, 1971). Leghemoglobin, an O_2-binding protein in the cytosol of the infected cells, is also likely to be affected by low temperatures as a result of both a lower diffusion coefficient for the oxygenated form of leghemoglobin (Moll, 1968) and a change in the affinity of leghemoglobin for O_2 and for other substrates (Imamura and Riggs, 1972; Stevens, 1982). The respiratory capacity of a nodule is also likely to be lower at low temperatures (Earnshaw, 1981).

On the basis of measured rates of respiration and assumptions concerning low *Oi*, nodule permeability to O_2 in lupin, soybean (*Glycine max*), and white clover has been estimated to increase with a temperature rise and decrease with a temperature drop (Minchin et al., 1986, 1992). Weisz and Sinclair (1988) measured nodule permeability to acetylene and ethylene diffusion in soybean, showed that it was correlated with temperature, and concluded that "the effects of temperature on N_2 fixation rate may be explained through an effect on nodule gas permeability." If this is correct, a decrease in nodule temperature may be expected to decrease nodule permeability such that nodule metabolism becomes more O_2 limited than it was at higher temperature. Also, an increase in the rhizosphere pO_2 should recover some or all of the initial nitrogenase activity. Similarly, an increase in nodule temperature should increase nodule permeability to O_2, thereby reducing the degree to which nitrogenase activity is limited by O_2.

The present study examined the role of O_2 in mediating the effects of temperature on nitrogenase activity in soybean nodules by studying both the time course of changes in

[1] Supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada research grant to D.B.L. and a NSERC postgraduate scholarship to M.M.K.

* Corresponding author; fax 1–613–545–6617.

Abbreviations: *ANA*, apparent nitrogenase activity; DW_{nod}, dry weight of nodules; DW_{nrt}, dry weight of nodulated root; *EAC*, electron allocation coefficient of nitrogenase; *F*, flux of O_2 into a nodule; *Oe*, atmospheric O_2 concentration in equilibrium with water at given temperature; *Oi*, infected cell O_2 concentration; OLC_N, the oxygen limitation coefficient of nitrogenase; *P*, permeability of a nodule to O_2 diffusion; pO_2, partial pressure of O_2; *PNA*, potential nitrogenase activity; Q_{10}, ratio of nodule activity at one temperature to that at a temperature 10°C lower; *TNA*, total nitrogenase activity.

nitrogenase activity and nodule respiration during temperature change and the effect of temperature on the degree to which the metabolism of soybean nodules was O_2 limited. In addition, studies were carried out on uninhibited and Ar:O_2-inhibited nodules to assess the effect of the initial O_2 status of the nodule on their temperature response.

MATERIALS AND METHODS

Plant Culture

Soybean plants (*Glycine max* L. Merr. cv Maple Arrow) were grown in silica sand in growth pots maintained in a controlled environment cabinet (Conviron Environments, Ltd., Winnipeg, Manitoba, Canada) at 20°C with a 16-h photoperiod (400 μmol quanta PAR m^{-2} s^{-1}) and 80% RH. Plants were irrigated twice daily with a nutrient solution containing 0.5 mM KNO_3 for the first 2 weeks after sowing. For the remaining growth period they were watered with N-free nutrient solution. Plants were inoculated 4 d after germination with *Bradyrhizobium japonicum* USDA 16, a strain lacking uptake hydrogenase activity (Layzell et al., 1984). Plants were used for experiments 28 to 32 d after inoculation, and all experiments were done while maintaining plants in an experimental growth cabinet with environmental conditions similar to growth conditions.

Effect of Temperature Change on Gas Exchange of Nodulated Roots

The night before each experiment, plants were moved into the experimental growth chamber and a thermister (YSI model 401, Yellow Springs, OH) was placed in the center of each pot. The pots were sealed and then flushed overnight (about 100 mL min^{-1}) with CO_2-free air. The next morning the pots were connected to an open-flow gas-exchange system (Hunt et al., 1989) containing a H_2 analyzer (model H-150, Morgan Instruments, Andover, MA) and an IRGA (model 225, Mark 3, Analytical Development Corp., Hoddesdon, UK). Pot temperature, nitrogenase activity (H_2 evolution), and respiration rate (CO_2 evolution) of the nodulated roots were measured continuously.

Plants were allowed to equilibrate in N_2:O_2 (80:20, v/v) at 20°C for 20 min and H_2 evolution was measured to provide an estimate of *ANA*. The atmosphere around the nodulated root was then changed to Ar:O_2 (80:20, v/v) and the peak H_2 evolution rate was determined as a measure of *TNA*1 in the nodules at 20°C. The gas around the nodulated roots was then immediately returned to a N_2:O_2 atmosphere and when a new steady-state *ANA* (*ANA*1) was obtained (about 10 min), pots were placed in a recirculating water bath maintained at either 15 or 25°C (only the pots were submerged in the water). Control plants were left at 20°C in the chamber. Preliminary tests showed that placing plants in a water bath at 20°C had no effect on nodule activity (data not shown). When a new steady-state *ANA* (*ANA*2) value was obtained at either 15 or 25°C (about 25 min), the atmosphere around the roots was changed again to Ar:O_2 and peak H_2 evolution rate was determined as a measurement of *TNA*2. Immediately after the measurement of *TNA*2, the O_2 concentration around the root was increased linearly from 20 to 100% at 2% O_2/min (O_2 ramp). *PNA* was determined as peak H_2 evolution in Ar:O_2 under conditions in which nodule metabolism was not limited by O_2 (Diaz del Castillo et al., 1992). An O_2 ramp of 2% O_2/min was chosen because it caused the highest stimulation of nitrogenase activity. The *EAC* (= 1 − [*ANA*/*TNA*]; Edie and Phillips, 1983) was calculated before (using *ANA*1 and *TNA*1) and after (using *ANA*2 and *TNA*2) the temperature change. The OLC_N (= *TNA*/*PNA*; Diaz del Castillo et al., 1992) was calculated for each temperature treatment and for the control plants (using *TNA*2 and *PNA*).

To study the effects of temperature change on O_2-limited nodules, nitrogenase activity was inhibited by prolonged exposure of nodulated roots to Ar:O_2 (80:20, v/v) before applying the temperature treatments described above. Initially *ANA* and *TNA*1 were measured, but instead of returning the nodulated roots to a N_2:O_2 environment, plants were left in Ar:O_2 until H_2 evolution declined (Ar-induced decline) to a new steady-state value (*TNA*2) (about 75 min). At this point the root temperature of experimental plants was changed to either 15 or 25°C, whereas control plants were left undisturbed at 20°C. H_2 evolution in Ar:O_2 was measured during each temperature treatment until a new steady state (*TNA*3) was reached. *PNA* was then measured by increasing pO_2 around the nodulated roots and OLC_N was calculated as *TNA*3/*PNA*. After the experiments, nodules were excised from the roots, and roots and nodules were washed to remove sand, dried separately at 85°C for 72 h, and weighed.

RESULTS

Effect of Temperature on Gas Exchange of Uninhibited Nodulated Roots

Plants of all three treatment populations (control 20°C, 25°C, and 15°C) had similar initial *ANA* (*ANA*1 = 79 ± 4 μmol H_2 g^{-1} DW_{nod} h^{-1}, n = 15) and *TNA* (*TNA*1 = 256 ± 6 μmol H_2 g^{-1} DW_{nod} h^{-1}, n = 15) values before the temperature treatments were imposed. In the control plants (Fig. 1A) there were no significant changes with time in *ANA*, *TNA*, or respiration rate. Increasing external pO_2 caused only a small stimulation of nitrogenase activity in control plants, such that *PNA* was not significantly different from *TNA*2 (Fig. 1A).

When the temperature of the nodulated roots was increased from 20 to 25°C (Fig. 1B), *ANA* and respiration rate increased gradually to 141 ± 5% (*ANA*2) and 132 ± 2%, respectively, of the values obtained before the temperature treatment. The *TNA* value at 25°C (*TNA*2) increased to 134% of the value at 20°C (*TNA*1). An increase in the external pO_2 caused only a small stimulation of nitrogenase activity, such that *PNA* was not significantly different from *TNA*2 (Fig. 1B).

When the temperature around the nodulated roots was decreased from 20 to 15°C (Fig. 1C), *ANA* first decreased to about 41 ± 8% of its initial value but then recovered to 59 ± 5% of that value (*ANA*2). Respiration rate gradually declined to 55 ± 2% of the initial value at 20°C and then remained stable. The *TNA*2 (at 15°C) was only 54% of the *TNA*1 (at 20°C), and *PNA* measured at 15°C was 111% of *TNA*2 but only 60% of *TNA*1. Therefore, the O_2 ramp was not able to recover the initial nitrogenase activity at 20°C.

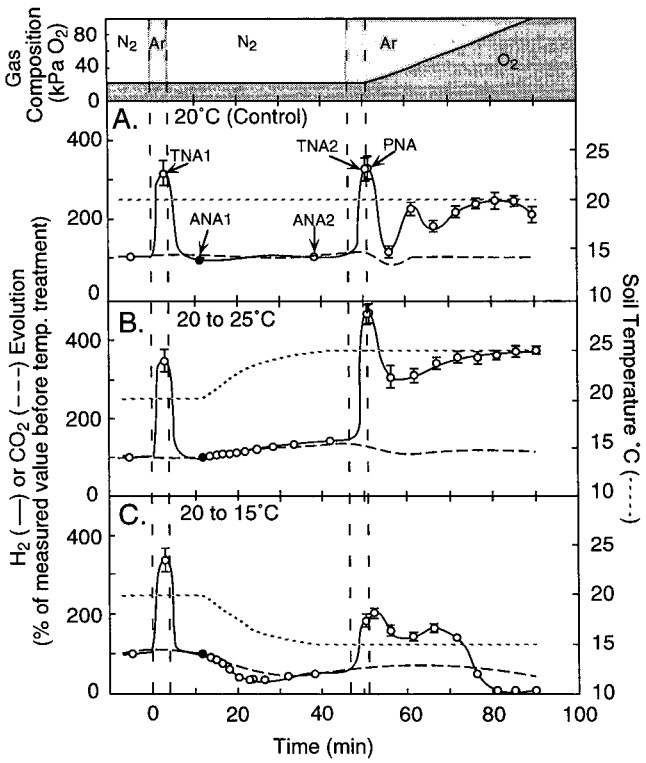

Figure 1. The time course of changes in H_2 evolution (—), CO_2 evolution (- - -) and rhizosphere temperature (· · · ·) in uninhibited plants at 20°C in which the pots were subsequently left at 20°C (A) or were transferred to 25°C (B) or 15°C (C). Traces pass through data points that represent the mean values ± SE of five replicate plants. SE values are included only where they exceed the points. Rates of H_2 evolution and CO_2 evolution are presented as a proportion of that value obtained just before the temperature treatment (●). These values before the temperature treatment for H_2 evolution (*ANA*1) were 84 ± 8, 75 ± 6, and 77 ± 7 μmol H_2 g^{-1} DW_{nod} h^{-1} for 20, 25, and 15°C treatments, respectively. Values for CO_2 evolution before the temperature treatments were 154 ± 15, 197 ± 44, and 137 ± 9 μmol CO_2 g^{-1} DW_{nrt} h^{-1} for 20, 25, and 15°C treatments, respectively. The uppermost panel of the figure shows the gas composition in the pot at specific times of the experimental run.

Effect of Temperature on Gas Exchange of Ar:O_2-Inhibited Nodulated Roots

Plants in all three treatment populations had similar initial *TNA* values (*TNA*1 = 222 ± 6 μmol H_2 g^{-1} DW_{nod} h^{-1}, n = 24), and prolonged exposure to Ar:O_2 caused this activity to decline to about 29% of this value (*TNA*2 = 64 ± 4 μmol H_2 g^{-1} DW_{nod} h^{-1}, n = 24) (Fig. 2). After the Ar-induced decline in nitrogenase activity, control plants showed no additional changes in their activity (compare *TNA*2 and *TNA*3 in Fig. 2A). The O_2 ramp caused a 2.9-fold increase in nitrogenase activity (*PNA*) relative to *TNA*3, but the *PNA* was only 82% of the *TNA*1.

When the temperature of the Ar:O_2-inhibited nodulated roots was increased from 20 to 25°C (Fig. 2B), *TNA* initially declined to 86 ± 2% of the value before the temperature treatment (*TNA*2), but then recovered to 143 ± 18% (*TNA*3) of that value. Respiration rate in these plants was stimulated to 144 ± 8% of the initial value with the temperature rise. The O_2 ramp that followed the high-temperature treatment resulted in a *PNA* value that was 2.2-fold higher than *TNA*3 and 114% of the *TNA*1.

When the temperature of the nodulated roots was decreased from 20 to 15°C (Fig. 2C), *TNA* initially increased to 107 ± 3% of the value before the temperature treatment (*TNA*2), but then declined to 69 ± 10% (*TNA*3) of that value. Respiration rate declined to 64 ± 2% of its initial value before the treatment. The O_2 ramp in these plants resulted in a *PNA* value that was 2.6-fold higher than *TNA*3 but only 38% of the *TNA*1.

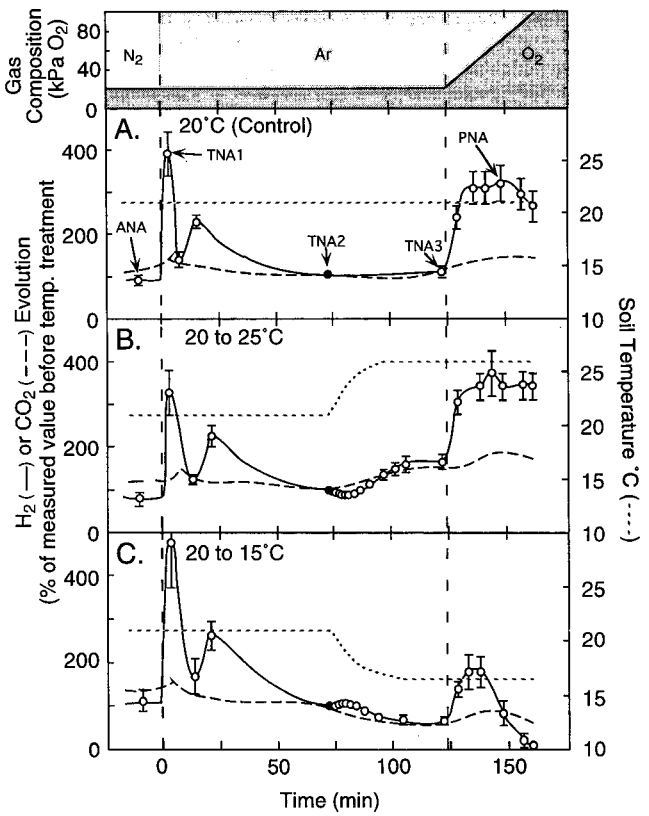

Figure 2. The time course of changes in H_2 evolution (—), CO_2 evolution (- - -), and rhizosphere temperature (· · · ·) in Ar:O_2-inhibited plants obtained as described in "Materials and Methods." Pots were left at 20°C (A) or were transferred to 25°C (B) and 15°C (C) after Ar:O_2 inhibition as described in "Materials and Methods." Traces pass through points of mean values from six replicate plants in B and C and 12 replicate plants in A. SE values are included only where they exceed the points. Rates of H_2 evolution and CO_2 evolution are presented as a proportion of that value obtained after Ar:O_2 inhibition and just before the temperature treatment (●). These values for H_2 evolution (*TNA*2) were 62 ± 6, 71 ± 7, and 62 ± 8 μmol H_2 g^{-1} DW_{nod} h^{-1} for 20, 25, and 15°C treatments, respectively. Values for CO_2 evolution after Ar:O_2 inhibition and before the temperature treatments were 213 ± 28, 188 ± 26, and 202 ± 18 μmol CO_2 g^{-1} DW_{nrt} h^{-1} for 20, 25, and 15°C treatments, respectively. The uppermost panel of the figure shows the gas composition in the pot at specific times of the experimental run.

Relative Changes in *ANA*, *TNA*, and *PNA* with Temperature

In uninhibited plants (Fig. 3A) a decrease in root temperature caused both *ANA* and *TNA* to decline by about 40%, and an increase in temperature caused an increase in *ANA* and *TNA* of about 40%, indicating a Q_{10} for nitrogenase activity of about 2.6. As a result, the *EAC* was not altered by the temperature treatment, being 0.67 ± 0.03 at 20°C, 0.68 ± 0.02 at 25°C, and 0.69 ± 0.04 at 15°C. An increase in root temperature of uninhibited plants caused a proportional increase in *TNA* and *PNA*. This resulted in no change in the OLC_N (0.99 ± 0.01) relative to that in control plants (0.99 ± 0.02; Fig. 3A). A decrease in temperature, however, caused a small decrease in OLC_N (0.91 ± 0.03) relative to that in control plants (Fig. 3A).

In Ar-inhibited plants, the low-temperature treatment caused a proportional reduction in both *PNA* and *TNA* (Fig. 3B) so that the OLC_N (0.39 ± 0.06) did not differ significantly from that of the control plants (0.33 ± 0.02). However, after the high-temperature treatment of Ar:O_2-inhibited plants, *TNA* increased relatively more than *PNA*, resulting in an OLC_N value (0.50 ± 0 .03) that was higher than that of the control plants (Fig. 3B).

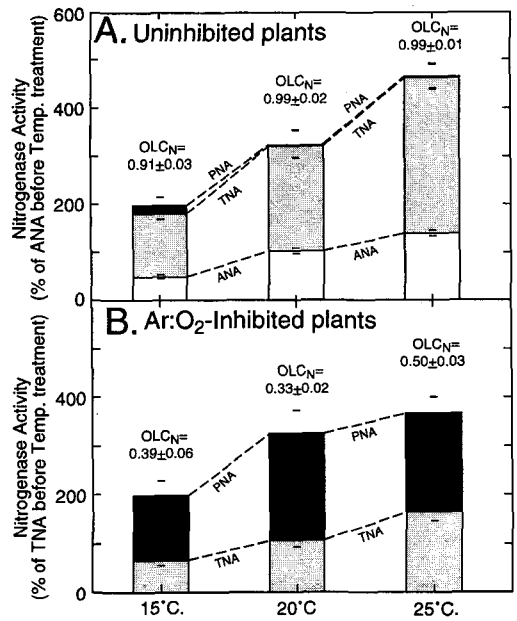

Figure 3. Nitrogenase activity measured as *ANA*, *TNA*, and *PNA* in uninhibited (A) and Ar:O_2-inhibited (B) nodules after they were exposed to a constant 20°C temperature (control), a change in temperature from 20 to 15°C, or a change from 20 to 25°C. The values for *ANA*, *TNA*, and *PNA* are plotted as overlaid bar graphs and are presented as percent of *ANA*1 (A) or *TNA*2 (B) values measured in the same plant prior to the temperature treatments (see Figs. 1 and 2). Values are presented as mean ± SE; n = 5 (A), n = 6 for 15°C and 25°C temperature treatments and n = 12 for control (20°C) treatment (B). The specific values of *ANA* and *TNA* before the temperature treatments are listed in the legends of Figures 1 and 2, respectively. The numbers at the top of each bar graph represent the OLC_N ± SE with replicate numbers as mentioned above for mean nitrogenase activities.

DISCUSSION

Does Temperature Affect the Degree of O_2 Limitation of Nodule Activity?

The OLC_N is a measure of the degree to which nitrogenase activity is limited by O_2. As in previous studies (King and Layzell, 1991; Diaz del Castillo et al., 1992; Kuzma et al., 1993), extended exposure of nodulated roots to Ar:O_2 caused a large decrease in OLC_N (from 0.99 to 0.33, Fig. 3, A and B), reflecting a greater degree of O_2 limitation in the Ar:O_2-inhibited nodules. Previous studies have shown that shoot removal, phloem deprivation, nodule detachment, and NO_3^- fertilization cause similar large decreases in OLC_N. For example, shoot removal caused a decrease in OLC_N from 0.79 to 0.47 in pea and from 0.81 to 0.44 in lupin (Diaz del Castillo et al., 1992). In soybean, OLC_N changed from 0.98 to 0.33 with stem girdling, to 0.47 with NO_3^- fertilization (calculated from Vessey et al., 1988), and to 0.22 with nodule detachment (calculated from Sung et al., 1991). By comparison, changes in the temperature of the nodulated root had only minor effects on OLC_N of soybean nodules even though the magnitude of nitrogenase inhibition by low temperature was similar to that observed after stem girdling, NO_3^- fertilization, and Ar:O_2 exposure. With a temperature decrease, OLC_N either did not change (Ar:O_2-inhibited nodules, Fig. 3B) or changed only from 0.99 to 0.91 (uninhibited nodules, Fig. 3A). Similarly, an increase in temperature had no effect on OLC_N (uninhibited nodules, Fig. 3A) or was associated with an increase in OLC_N from 0.33 to 0.50 (Ar:O_2-inhibited nodules, Fig. 3B).

These data indicate that the mechanism by which legume nodules adapt their O_2 status to changes in temperature differs from that associated with other environmental treatments such as phloem deprivation, nodule detachment, NO_3^- fertilization, or Ar:O_2 exposure. Apparently, O_2 limitation plays only a minor role in the regulation of nitrogenase activity during changes in temperature.

O_2 Limitation of Nodule Activity and Nodule Permeability

The P (m s^{-1}) can be estimated from specific nodule respiration rates using Fick's law of diffusion (Sheehy et al., 1983; Minchin et al., 1986; Vessey et al., 1988):

$$P = \frac{F}{(Oe - Oi)} \quad (1)$$

where F is the flux of O_2 into the nodule (units of mol m^{-2} s^{-1}), Oe is the concentration of dissolved O_2 (units of mol m^{-3}) in the aqueous phase of the cortical spaces at 20 kPa O_2, and Oi is the infected cell O_2 concentration (assumed to be zero for these calculations). To obtain estimates of nodule permeability to O_2 in the present study, a measure was needed for values of F in the nodules at each temperature. Values for F were calculated from *TNA* values using a relationship between specific nodule respiration and *TNA* (Fig. 4) obtained in a previous study (Kuzma et al., 1993) with

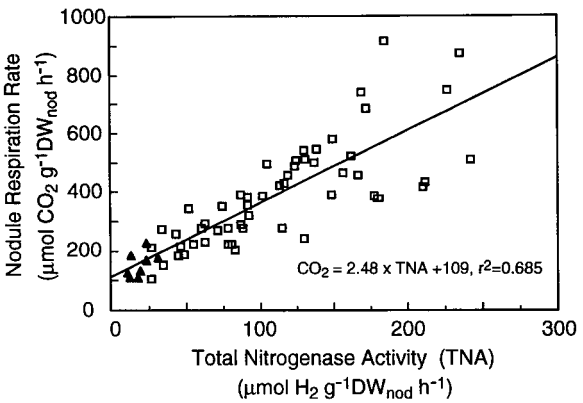

Figure 4. Relationship between the rate of respiratory CO_2 evolution and the *TNA* of single, intact soybean nodules as measured using a single nodule probe. The data were from a previous study of soybean nodules (figure 3 and table I of Kuzma et al., 1993) maintained at 22°C in which measurements were made of CO_2 evolution and peak H_2 evolution in an Ar:O_2 atmosphere (□) or CO_2 evolution and steady-state H_2 evolution in Ar:O_2 (after Ar:O_2 inhibition of nitrogenase activity) (▲). The line and equation on the figure represent a linear regression of the data in which the *y* intercept (growth and maintenance respiration) was 109 μmol CO_2 g^{-1} DW_{nod} h^{-1}, and the slope (respiration associated with each unit of TNA) was 2.48 μmol CO_2 μmol^{-1} H_2. These values were similar to those obtained in previous studies of the respiratory cost of N_2 fixation in soybean nodules (Witty et al., 1983; Ikeda et al., 1992).

single, attached nodules of the same symbiosis as that used in the present study. This previous study (Kuzma et al., 1993), carried out at 23°C, was used to obtain a value of 109 μmol CO_2 g^{-1} DW_{nod} h^{-1} as a measure of the respiration associated with nodule growth and maintenance, and a value of 2.48 μmol CO_2 μmol^{-1} H_2 as a measure of the respiration associated with each unit of TNA. These values were similar to those obtained in previous studies of the respiratory cost of N_2 fixation in legume nodules (Witty et al., 1983; Ikeda et al., 1992). To convert these values to rates of O_2 uptake (*F*) in the nodules equilibrated to 15, 20, and 25°C, it was assumed that a measure of respiration rate associated with each unit of TNA is independent of temperature, since both respiration rate and nitrogenase activity are affected by temperature to the same degree (Q_{10} of 2.6). The growth and maintenance respiration was assumed to have a temperature coefficient (Q_{10} of 2.0; Amthor, 1984), and the RQ of 1.1 CO_2 per O_2 (Minchin and Witty, 1990) was used. The resultant values (Table I) indicated that at 25°C, O_2 flux was 1.3 to 1.8 times higher than at 20°C, and at 15°C the flux was 0.55 to 0.69 times that at 20°C. When these values for *F* were used to calculate *P*, it was estimated that an increase in temperature from 20 to 25°C was associated with a 61 and 118% increase in *P* in uninhibited and Ar:O_2-inhibited nodules, respectively, whereas a decrease in temperature from 20 to 15°C caused a decrease in *P* to 53 and 67% of that at 20°C in uninhibited and Ar:O_2-inhibited nodules, respectively. Changes of similar magnitude were observed by Weisz and Sinclair (1988), who used an ethylene lag phase assay to measure *P* in uninhibited soybean nodules after changes in temperature between 20, 24, and 28°C.

Because only small or insignificant changes in OLC_N were

Table I. *The effect of temperature on the flux of O_2 (units of mol m^{-2} s^{-1}) and the permeability of O_2 diffusion (P, units of m s^{-1}) in uninhibited and Ar:O_2-inhibited soybean nodules*

Values are presented as mean ± SE.

Treatments	Temperature	TNA[a]	Sample Size	Nodule Respiration[b]	O_2 Flux[c]	P^d
	°C	μmol g^{-1} DW_{nod} h^{-1}	*n*	μmol g^{-1} DW_{nod} h^{-1}	μmol m^{-2} s^{-1}	μm s^{-1}
Uninhibited nodules	15	140 ± 11	5	413 ± 16	11.0 ± 0.4	36.4 ± 2.3
	20	226 ± 7	5	754 ± 17	20.1 ± 0.4	67.8 ± 1.5
	25	347 ± 12	5	993 ± 26	26.4 ± 0.8	109.3 ± 3.3
Ar:O_2-inhibited nodules	15	39 ± 6	6	165 ± 15	4.4 ± 0.4	14.5 ± 1.3
	20	59 ± 4	12	241 ± 9	6.4 ± 0.2	21.7 ± 0.8
	25	120 ± 10	6	431 ± 24	11.5 ± 0.7	47.4 ± 2.7

[a] *TNA*2 values obtained from Figure 1 and *TNA*3 values obtained from Figure 2. [b] For plants at 20°C, values were calculated from the measured *TNA* values using the linear regression of Figure 4, where growth and maintenance respiration (the *y* intercept of the regression line of Fig. 4) was calculated to be 94.5 μmol g^{-1} DW_{nod} h^{-1} assuming a Q_{10} for growth and maintenance respiration to be 2.0 (Amthor, 1984). At 15 and 25°C, values were calculated in the same way except that growth and maintenance respiration was calculated to be 66.8 and 133.7 μmol g^{-1} DW_{nod} h^{-1}, respectively. [c] Calculated from the nodule respiration rate assuming a respiratory quotient of 1.1 CO_2 per O_2 (Minchin and Witty, 1990), and 28.5 mm^2 of nodule surface area for a typical spherical nodule having a diameter of 4 mm (Vessey et al., 1988). [d] Calculated from Equation 1 (see text) given the value for O_2 flux derived in this table, *Oe* values of 0.296, 0.242, and 0.302 mol m^{-3} were used (O_2 concentrations in water at equilibrium with 20 kPa O_2 at 20, 25, and 15°C, respectively, calculated from solubilities of O_2 at these temperatures; Altman and Dittmer, 1971), *Oi* was assumed to be zero.

observed after the low-temperature treatment in both uninhibited and Ar:O_2-inhibited nodules, the decrease in *P* is more likely to be a result of temperature change than the cause of nitrogenase inhibition. Similar effects have recently been observed with water-stressed soybean nodules (Diaz del Castillo et al., 1993) in that a decrease in nitrogenase activity and *P* occurred without an associated change in the OLC_N.

During the temperature increase, no change in OLC_N was observed in uninhibited nodules, but a small increase in OLC_N value was observed in Ar:O_2-inhibited nodules. Also, *P* increased to a greater extent in Ar:O_2-inhibited nodules than in uninhibited nodules. Although no stimulatory environmental treatments have been identified that are comparable to high temperature, it is clear that the degree of O_2 limitation of nitrogenase before the temperature treatment affects subsequent changes in OLC_N.

O_2 Limitation of Nodules and the Response of Nitrogenase Activity to Temperature Change

The results of the present study showed that the initial O_2 status of the nodules greatly influenced the effect of temperature on OLC_N. The Ar:O_2 treatment also affected the time course of change in nitrogenase activity following the temperature change. Figure 5 shows the relative changes in *ANA* (uninhibited nodules) and *TNA* (Ar:O_2-inhibited nodules) as temperature was increased or decreased. A rise in temperature from 20 to 25°C resulted in a gradual increase in nitrogenase activity of the uninhibited nodules to about 140% of the value at 20°C, whereas the activity in Ar:O_2-inhibited nodules initially declined and then rose to a level similar to that in the uninhibited nodules.

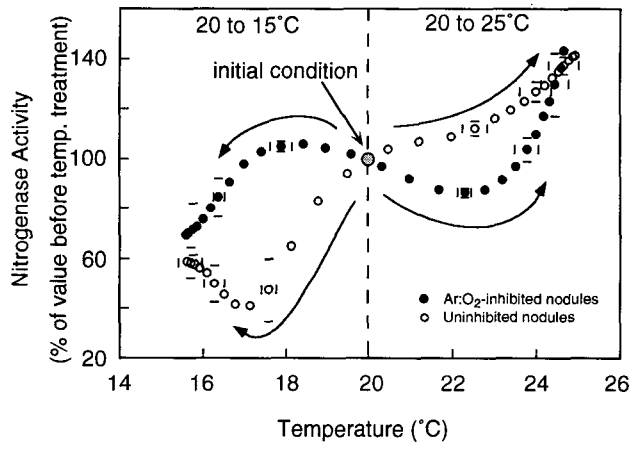

Figure 5. Responses of nitrogenase activity to temperature treatments in uninhibited nodules (O) and Ar:O_2-inhibited nodules (●). Nitrogenase activity is expressed as percent of *ANA*1 value in uninhibited nodules and percent of *TNA*2 value in Ar:O_2-inhibited nodules. Points are taken every 2 min from the start of the temperature treatments and they represent mean nitrogenase activities ± SE (n = 5 or 6) for the mean temperature at each time interval. SE bars are included on every fourth point and the arrows represent the time course of the change in nitrogenase activity with temperature.

In contrast, a decline in temperature from 20 to 15°C in the uninhibited nodules resulted in a rapid decrease in nitrogenase activity followed by a recovery to a new value at about 60% of the value at 20°C. In the Ar:O_2-inhibited nodules, nitrogenase activity was initially stimulated, but it subsequently decreased to a new value at about 69% of the value at 20°C. Therefore, despite the fact that the uninhibited and Ar:O_2-inhibited nodules differed in the response of nitrogenase activity to temperature change, similar final relative nitrogenase activities were observed in nodules from both treatments following a similar increase or decrease in temperature.

The results shown in Figure 5 may be accounted for in two distinct ways. First, it is possible that the different responses may reflect the fact that nitrogenase activity was measured as *ANA* in the uninhibited nodules and as *TNA* in the Ar:O_2-inhibited nodules. If the allocation of electrons between N_2 fixation and H_2 evolution were affected by the temperature treatments, this might explain the differences observed in the time courses of nitrogenase activity. Previous studies have reported that *EAC* of nitrogenase increases at low temperature and decreases at high temperature (Rainbird et al., 1983; Layzell et al., 1984; Pankhurst and Layzell, 1984; Bertelsen, 1985). However, in the present study *EAC* remained unchanged after the temperature treatments. The reasons for this discrepancy are not fully understood, although it has been suggested (Hunt and Layzell, 1993) that the results of some of the previous studies may be artifactual due to problems in the measurement of nitrogenase activity by the acetylene reduction assay.

Nevertheless, for changes in *EAC* to account for the results shown in Figure 5 these changes would have to occur only during the temperature change, since *EAC* values at the end of the temperature treatments were similar to initial values at all three temperatures (Fig. 3). Moreover, *EAC* during the temperature changes would have to increase to 0.89 (calculations not shown), a value that is much higher than the maximal theoretical *EAC* of 0.75 (Burris, 1985). Although this seems unlikely, direct support or rejection of this hypothesis will require measurements of *EAC* during the temperature treatment, a difficult prospect considering the rapidity of the changes in nitrogenase activity.

The second explanation to account for different responses of nitrogenase activity to temperature change (Fig. 5) assumes that the *ANA* measurements in the uninhibited nodules, and the *TNA* measurements in the Ar:O_2-inhibited nodules reflect changes in total electron flow through nitrogenase. The proposed mechanism of nodule acclimation to temperature change based on this assumption is outlined in the following section.

A Proposed Mechanism for Nodule Acclimation to Changing Temperature

The results of this study indicate that temperature affects the respiratory capacity of the nodule. Changes in respiratory capacity may result in changes in *Oi*, and we propose that nodules acclimatize to changing temperature by responding to altered nodule O_2 status.

The proposed sequence of events during the temperature

decrease is as follows: (a) At low temperature O_2 consumption by the bacteroids as well as the plant fraction decreases, resulting in higher *Oi*. (b) The increase in *Oi* causes initial inhibition of nitrogenase activity in control (uninhibited) nodules, but nodules that were severely O_2 limited (Ar:O_2-inhibited) before the temperature decrease experience initial stimulation of nitrogenase activity. (c) *P* decreases as a result of increased *Oi*, limiting additional O_2 entry into the nodule. (d) *Oi* declines as O_2 accumulated in the infected cells is consumed. (e) Nitrogenase activity recovers as *Oi* returns to initial noninhibitory levels. (f) Further reduction of *Oi* to suboptimal levels for nitrogenase activity may occur with further reduction in nodule permeability.

The gas-exchange data support this sequence of events. Low temperature results in significant decreases of nodulated root respiration rates (Figs. 1 and 2), which should cause *Oi* to increase (prediction a). Rapid inhibition of nitrogenase activity in uninhibited nodules (Fig. 5) occurs immediately after the onset of the low-temperature treatment. Also, nitrogenase activity of O_2-limited nodules is initially stimulated (Fig. 5) by a temperature decrease (prediction b). Also, only a small or insignificant decrease in OLC_N occurred after the low-temperature treatment (Fig. 3) despite a significant decline in nodule permeability to O_2 (Table I). This indicates that at the end of the low-temperature treatment, *Oi* was not much different from its initial value at 20°C (predictions c, d, and f). Initial inhibition of nitrogenase activity was followed by some recovery in uninhibited nodules (Fig. 5) (prediction e).

The proposed sequence of events during temperature increase is as follows: (a) At high temperature the demand for O_2 by the bacteroids increases, resulting in an initial decline in *Oi*. (b) This decline in *Oi* results in delayed stimulation of nitrogenase activity by increased temperature of control nodules, whereas in severely O_2-limited nodules nitrogenase activity is inhibited. (c) *P* increases to allow more O_2 to enter the nodule, resulting in a recovery of *Oi* to initial levels or an increase in *Oi* to above initial levels. (d) O_2 consumption rates are stimulated by increased *Oi* and nitrogenase activity is stimulated.

Again, the gas-exchange data support this sequence of events. With increased temperature, nodulated root respiration rates increased (Figs. 1 and 2) (prediction a). Nitrogenase activity of uninhibited nodules increased gradually and that of severely O_2-limited nodules was initially inhibited (prediction b). Nodule permeability increased significantly (Table I) after the temperature increase, but an increase in OLC_N was observed only in severely O_2-limited nodules (Fig. 3B) (prediction c). Increased OLC_N would not be apparent in uninhibited nodules because OLC_N in these nodules is already close to unity. Prediction d is supported by data in Figure 5 that show stimulation of nitrogenase activity in both uninhibited and Ar:O_2-inhibited nodules to the same extent.

To test these proposed mechanisms of nodule acclimation to changing temperature, it will be necessary to monitor *Oi* in both uninhibited and severely O_2-limited nodules exposed to increases or decreases in temperature. This will be the topic of a subsequent paper.

Received December 27, 1993; accepted May 13, 1994.
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