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Adaptation of Nitrogen Fixation by Intact Soybean Nodules to Altered Rhizosphere pO_2[1]

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ABSTRACT

The N_2-fixing legume nodule requires O_2 for ATP production; however, the O_2 sensitivity of nitrogenase dictates a requirement for a low pO_2 inside the nodule. The effects of long term exposures to various pO_2s on $N_2[C_2H_2]$ fixation were evaluated with intact soybean (*Glycine max* [L.] Merr., var. Wye) plants. Continuous exposure of their rhizosphere to a pO_2 of 0.06 atmospheres initially reduced nitrogenase activity by 37 to 45% with restoration of original activity in 4 to 24 hours and with no further change in tests up to 95 hours; continuous exposure to 0.02 atmosphere of O_2 initially reduced nitrogenase activity 72%, with only partial recovery by 95 hours. Similar exposures to a pO_2 of 0.32 atmospheres had little effect on $N_2[C_2H_2]$ fixation; a pO_2 of 0.89 atmospheres initially reduced nitrogenase activity by 98% with restoration to only 14 to 24% of that of the ambient O_2 controls by 95 hours. Re-exposure to ambient pO_2 of plants adapted to nonambient pO_2s reduced $N_2[C_2H_2]$ fixation to similar magnitudes as the reductions which occurred upon initial exposure to variant pO_2 conditions, and a time period was required to readapt to ambient O_2. It is concluded that the $N_2[C_2H_2]$-fixing system of intact soybean plants is able to adapt to a wide range of external pO_2s as probably occur in soil. We postulate that this occurs through an undefined mechanism which enables the nodule to maintain an internal pO_2 optimal for nitrogenase activity.

Oxygen is essential for ATP production by oxidative phosphorylation to sustain the symbiotic N_2 fixation process in legume nodules; however, the nitrogenase enzyme is remarkably sensitive to O_2 with rapid inactivation occurring upon exposure to ambient O_2 concentrations (4). Recent research findings reveal the systems used by the legume nodule to satisfy these O_2 needs. It has been suggested (4) and there is evidence (6, 23) that leghemoglobin in the soybean (*Glycine max* [L.] Merr.) nodule facilitates a high O_2 flux to the bacteroids at a very low O_2 concentration estimated (2, 23) to range from 0.005 to 0.010 mm Hg, or lower. Direct measurements of the pO_2 in the central tissue of the nodule show extremely low values and provide evidence for a barrier in the inner part of the nodule cortex which limits O_2 diffusion from the external environment (22). Other researchers suggest that there is a continuous network of intercellular spaces which permits gaseous diffusion of O_2 from the external atmosphere to the host cells of the cortex, the interstitial cells, and the mitochondria at the periphery of the bacteroid-containing cells, all of which represent a continuum of O_2 sinks (5).

However, in spite of these elaborate mechanisms, the N_2-fixing activity of the soybean nodule appears to be limited by the pO_2 in air since maximal rates of N_2 fixation, measured in short term experiments using excised nodules, usually are increased by supra-ambient pO_2s (3, 7). A comprehensive study, using field-grown plants throughout the complete growth cycle, gave maximal rates of N_2 fixation at 0.26 to 0.41 atm O_2 with substantial decreases at subambient pO_2s (9).

In the soil environment of the nodule, pO_2s greater than 0.21 atm probably do not occur naturally, while subambient pO_2s probably exist under various environmental circumstances (4, 17, 21). Thus, an ideal legume N_2-fixing system should be able to adapt rapidly to a range of subambient pO_2s such as occur in soil so as to maximize N_2 fixation. The aerobic N_2-fixing bacterium *Azotobacter* possesses mechanisms that protect its nitrogenase from O_2 extremes (18), but based on previous research findings (3, 7), it would appear that agriculturally important legumes cannot successfully cope with pO_2 fluctuations in the rhizosphere. Through the use of long term, intact plant studies, we have discovered and report here that the N_2-fixing system in the soybean nodule can adapt to such a variant range of rhizosphere pO_2s. A preliminary report of this work has been presented (9).

MATERIALS AND METHODS

Plant Culture. Two long term experiments were conducted to study $N_2[C_2H_2]$ fixation responses by nodules exposed to various rhizosphere pO_2s, and culture of the intact Wye soybeans used in both of the experiments was similar. Seeds treated with Agway, peat-based *Rhizobium japonicum* inoculum were planted in sterile silica sand contained in 18-cm diameter pots modified to serve as incubation vessels (8, 19) for assays of $N_2[C_2H_2]$ fixation. Seedlings emerged after 5 days. The seedlings were inoculated again with 50 ml of a 20% (v/v) peat-based inoculant drench to further insure nodulation and they were thinned to one plant/pot 1 week after planting. The plants were supplied with 150 ml/pot of N-free, Hoagland nutrient solution (14) on alternate days and deionized water on other days. In the first experiment, 200 ml/pot of full strength, complete Hoagland solution were applied once 2 weeks after planting, and in the second experiment, a 200 ml/pot application of 10% (v/v), complete Hoagland solution was applied 3 weeks after planting to provide the plants with a N source until the nodules became functional. The soybeans were grown in a controlled environment room having a 24/18 C day/night temperature cycle, a 46,300 lux, 12-hr photoperiod, and a 75% relative humidity. Exposures of the nodulated intact roots to variant pO_2s were commenced when the plants were 42 days of age and in the pod-elongation stage of development.

Assay for $N_2[C_2H_2]$ Fixation. In both experiments, the acetylene-ethylene procedure (13) was used to measure $N_2[C_2H_2]$-

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fixing activity. Intact plant incubations for measurement of nitrogenase activity were similar to those previously described (8, 19), and after 15 and 30 min of incubation, 10-cm³ gas samples were withdrawn and placed into 10-ml evacuated blood sample tubes (11) for subsequent gas chromatographic assay (8, 13) of C_2H_2 and C_2H_4. Because the pC_2H_2 used in the first and second experiments was insufficient to saturate nitrogenase (0.026 and 0.028 atm, respectively) $N_2[C_2H_2]$ fixation rates were adjusted to maximum velocity using the relationship $Vm = V + (VKm/[S])$ where V is the velocity of the reaction at substrate concentration $[S]$ and Km is the average reported Michaelis-Menten constant of 0.006 atm of C_2H_2 for nitrogenase (12). Incubation vessels contained from 775 to 710 cm³ of free air space in the first and second experiments, respectively. At field capacity conditions under which all assays were performed, the moisture content of the sand averaged 15.2%, and solubilities of C_2H_2 and C_2H_4 were considered in rate computations of $N_2[C_2H_2]$ fixation.

Experimental Design. Four rhizosphere pO_2s were used in the first long term study to evaluate pO_2 effects on $N_2[C_2H_2]$ fixation by nodulated intact plants. Exposure to a desired pO_2 was accomplished by purging $N_2|O_2|CO_2$ gas mixtures through the incubation vessels from top to midside port at a rate of 200 cm³/min between assays. Concentrations of O_2 in the $N_2|O_2|CO_2$ purge streams were measured twice daily with an O_2 electrode, and pO_2s averaged 0.06 ± 0.0, 0.21 ± 0.0, 0.32 ± 0.01, 0.89 ± 0.01 atm for the four treatments. No attempt was made to control the pCO_2 in the purge mixtures of the first experiment; however, calculations based on gas dilutions indicated that the pCO_2 ranged from approximately 65 to 320 μl/l for the various treatments. The pO_2 of 0.06 atm was obtained by addition of N_2 to air, whereas the pO_2s of 0.32 and 0.89 atm were obtained by addition of O_2 to air. Incubations for measurement of $N_2[C_2H_2]$ fixation at the four O_2 concentrations were performed at 24 C in different $Ar|O_2|C_2H_2$ gas mixtures when the experiment was initiated and after purging with the equivalent O_2-containing $N_2|O_2|CO_2$ gas mixture for average times of 3.9, 23.7, and 47.4 hr. Initially, there were four replications of each of the four pO_2 treatments, but following each incubation for measurement of $N_2[C_2H_2]$ fixation, with the exception of the ambient pO_2 treatment, one plant of each nonambient pO_2 treatment was immediately reincubated under ambient O_2 conditions. Subsequent purgings and $N_2[C_2H_2]$ fixation assays on these plants were conducted under ambient O_2 conditions for the duration of the study to test the reversibility of the previously imposed variant pO_2 treatment effects. After the $N_2[C_2H_2]$ fixation assays were completed, the silica sand was gently washed from the roots and nodules, the nodules were stripped from the roots, and the nodule fresh weight/plant was recorded. The data were expressed on a specific activity basis (μg $N_2[C_2H_2]$ fixed/g nodule fresh weight·hr). To test pO_2 treatment effects, the $N_2[C_2H_2]$ fixation specific activity data were subjected to a $\sqrt{X}$ transformation to obtain homogeneous data, and a completely random analysis of variance with unequal replication was performed. Transformed treatment means were compared by Duncan's new multiple range test as modified by Kramer for unequally replicated treatments (20).

The second long term adaptation study was similar to the first except that longer times of exposure to purge mixtures containing three different pO_2s were used between $N_2[C_2H_2]$ fixation incubations, and the balanced experiment was replicated five times. The partial pressure of O_2 in the continuous $N_2|O_2|CO_2$ purge mixtures averaged 0.02, 0.06, and 0.21 atm as measured with an O_2 electrode. The pCO_2 in the purge mixtures was controlled in the range from 333 to 339 μl/l, as determined by IR gas measurements. Initial assays for $N_2[C_2H_2]$ fixation were performed at 24 C in three $Ar|O_2|C_2H_2$ gas mixtures with pO_2s comparable to those of the purge gas mixtures, and subsequent assays were conducted in each of the three pO_2 treatments after continuous purging with equivalent O_2-containing $N_2|O_2|CO_2$ gas mixtures for 4, 23.9, 47.3, 71, and 95 hr. After continuous exposure to the three pO_2s for 99 hr, the intact plants were reincubated in ambient O_2 to test the reversibility of previous pO_2 adaptation effects. To confirm that pO_2s during $N_2[C_2H_2]$ fixation assays were near those of the treatment purge and incubation gas mixtures, 2-cm³ gas samples were withdrawn with a gas-tight syringe from the rhizosphere of four replicates of the three pO_2 treatments at the start and end of the 30-min incubations conducted after 71 hr. The O_2 content of the rhizosphere during incubation was determined with a Perkin Elmer 900 gas chromatograph equipped with a thermal conductivity detector and two specially pretreated (15) stainless-steel columns (2.7 m × 0.3 cm diameter) with a molecular sieve 5A packing connected in series and operated at 11 C. At the end of the experiment, the sand was washed from the roots and nodules, the nodules were stripped from the roots, and the nodule fresh weight was recorded enabling $N_2[C_2H_2]$ fixation rates to be computed on a specific activity basis. Data collected to assess the reversibility of long term pO_2 effects were analyzed separately from the variant pO_2 exposure data; however, both sets of data were subjected to $\sqrt{X}$ transformations and analyzed as factorial experiments to test pO_2 treatment and pO_2 incubation time effects and the interaction of these two factors. Duncan's new multiple range test was used to compare treatment means.

RESULTS AND DISCUSSION

The rate of $N_2[C_2H_2]$ fixation (Table I) was reduced 37% from the ambient O_2 control rates in intact plants following initial exposure of the rhizosphere to a subambient pO_2 of 0.06 atm, but after 4 hr of continuous exposure to this low pO_2, the $N_2[C_2H_2]$ fixation specific activities were restored and remained at levels comparable to those of the ambient O_2 controls throughout the remainder of the 2-day exposure. The $N_2[C_2H_2]$ fixation specific activities of the ambient O_2 control plants did not differ significantly over the 2-day study. Nitrogenase activity appeared to be slightly, although not significantly, reduced following exposure to 0.32 atm of O_2, and continuous exposure for 2 days gave activity indistinguishable from the controls. Initial incubations conducted in 0.89 atm of O_2 severely reduced the $N_2[C_2H_2]$ fixation specific activity, and subsequent exposure to this supra-ambient pO_2 resulted in a 10-fold recovery (Table I, read horizontally) of nitrogenase activity. However, the absolute recovery values of $N_2[C_2H_2]$ fixation in 0.89 atm of O_2 were only 14 to 24% of those of the ambient controls, and this lack of total recovery probably was associated with the O_2 sensitivity of the nitrogenase enzyme.

Re-exposure of the rhizosphere of intact nodulated plants to the ambient pO_2 initially reduced $N_2[C_2H_2]$ fixation in those treatments where the intact plants had been previously exposed to pO_2s other than ambient. The reductions in nitrogenase activity upon re-exposure to ambient O_2 were particularly evident in plants previously exposed to a pO_2 of 0.06 atm for 24 and 48 hr, and reductions in nitrogenase activity were not as evident in the supra-ambient pO_2 treatments (Table I, read vertically). Results of these studies also indicated that a time period was required for re-establishment of the control rate of $N_2[C_2H_2]$ fixation upon re-exposure of the intact plant to ambient O_2.

The second experiment was conducted to examine: (*a*) the effect of subambient O_2 concentrations over a longer exposure period; and (*b*) the range of subambient O_2 concentrations to which the nodule can adapt. The lowest rhizosphere pO_2 was 0.02 atm, and it was provided to determine if $N_2[C_2H_2]$ fixation adaptation would still occur under conditions where oxidative phosphorylation and ATP production rates would be expected to be more severely reduced initially. Specific activities of $N_2[C_2H_2]$

fixation were reduced 72% below the ambient O_2 controls when the nodulated roots of intact plants were first incubated with the rhizosphere containing a pO_2 of 0.02 atm (Table II). Plants continuously exposed to a pO_2 of 0.02 atm only partially recovered their nitrogenase activities, and with the exception of the incubation performed after 3 days, all subsequent $N_2[C_2H_2]$ fixation rates were below those of the ambient O_2 controls. Significant reductions of 45% in nitrogenase activity occurred when the intact plants were initially flushed and incubated with the 0.06-atm O_2 gas mixture, but after 1 day of continuous exposure to this subambient pO_2, the inhibitory effects of low O_2 were overcome, and subsequent $N_2[C_2H_2]$ fixation specific activities were comparable to those of ambient O_2 controls. As in the first experiment, $N_2[C_2H_2]$ fixation specific activities of the ambient O_2 control plants did not differ during the study. The pO_2 treatment × pO_2 incubation time interaction was highly significant because of the differential pO_2 treatment adaptation responses.

The adaptation of the $N_2[C_2H_2]$ fixation system to variant pO_2s was further verified by reincubating in ambient O_2 the intact plants that had been exposed to the two variant and ambient O_2 concentrations for 99 hr. When incubated in ambient pO_2, $N_2[C_2H_2]$ fixation specific activities of plants previously exposed to pO_2s of 0.02 and 0.06 atm were only 29 and 55% of respective 95-hr specific activities, and respective 99-hr nitrogenase activities were only 15 and 58% of those of the ambient O_2 controls (Table III). The 95- and 99-hr ambient O_2 control nitrogenase activities did not differ at the 5% level. Such results again confirmed that sensitivity of $N_2[C_2H_2]$ fixation activity developed upon re-exposure to ambient O_2 in intact plants conditioned, or partially conditioned, to subambient pO_2s. Sensitivity upon re-exposure to ambient O_2 was greatest in those treatments where the rhizosphere pO_2 deviated most from ambient, which resulted in a significant pO_2 treatment × pO_2 incubation time interaction.

Gas chromatographic measurements demonstrated that pO_2s averaged 0.02, 0.07, and 0.20 atm at the start and 0.03, 0.07 and 0.21 atm at the end of the 30-min $N_2[C_2H_2]$ fixation incubations for the low, intermediate, and ambient pO_2 treatments, respectively. These O_2 concentrations averaged slightly higher than those measured for the pO_2 component of the $Ar|O_2|C_2H_2$ incubation gas mixture; however, the measurements confirmed that the pO_2s in the rhizosphere were within 0.01 atm of those of the variant pO_2 purge streams. The fact that the O_2 concentrations remained stable throughout the incubation period indicated that root and nodule respiration did not consume significant quantities of O_2, and that the incubation vessels were effective in preventing exchange of O_2 from the external environment with that of the rhizosphere. In addition, it has been demonstrated that in soybeans, ^{15}O-labeled O_2 movement from the shoot to the roots does not occur through the stem (16).

We believe that the adaptation response of the N_2 fixation

Table I. *Specific Activities of $N_2[C_2H_2]$ Fixation by Intact Soybean Roots following Various Times of Exposure to Sub- and Supraambient Rhizosphere pO_2s*

pO_2	Mean Time from 0 hr until Reincubation in 0.21 atm O_2	Mean Incubation Times for Plants Continuously Exposed to Indicated pO_2's (hr)			
		0.0	3.9	23.7	47.4
atm	hr	μg $N_2[C_2H_2]$ fixed/g nodule fresh wt·hr			
0.06		78.6 e*	83.6 de	137.1 abcd	205.7 ab
	0.6	(93.8)[1]			
	4.1	(148.4)	(131.4)		
	23.8	(164.4)	(116.4)	(48.7)	
	47.8	(198.4)	(151.5)	(112.4)	(113.8)
0.21 (Control)		124.1 bcd	112.1 cde	150.9 abc	157.8 abc
0.32		92.7 de	146.5 abc	181.6 ab	228.1 a
	0.6	(83.9)			
	4.1	(118.1)	(84.9)		
	24.0	(159.4)	(156.8)	(148.3)	
	47.8	(165.5)	(145.2)	(228.5)	(174.9)
0.89		2.4 g	26.5 f	27.0 f	21.6 f
	0.6	(2.4)			
	4.1	(22.1)	(37.4)		
	23.8	(38.2)	(25.7)	(20.0)	
	47.8	(67.3)	(112.5)	(43.5)	(22.6)

*Numbers followed by the same letter do not differ at the 5% level by Duncan's new multiple range test as modified by Kramer for unequal replication.

[1]Numbers shown in parentheses are nonreplicated $N_2[C_2H_2]$-fixation specific activities by intact soybean plants previously exposed to nonambient O_2 conditions and reincubated in ambient O_2.

Table II. *Specific Activities of $N_2[C_2H_2]$ Fixation by Intact Soybean Roots following Various Times of Exposure to Subambient Rhizosphere pO_2s*

pO_2	Mean Incubation Times for Plants Continuously Exposed to Indicated pO_2's (hr)									
	0.0		4.0	23.9		47.3		71.0		95.0
atm	μg $N_2[C_2H_2]$ fixed/g nodule fresh wt·hr									
0.02	26.2	fg*	19.3 g	36.6	efg	47.0	def	73.0	bcd	65.2 cde
0.06	51.8	de	66.6 cd	134.2	a	140.2	a	130.8	a	137.2 a
0.21	94.8	abc	115.7 a	110.2	ab	114.9	a	108.1	ab	113.1 a

*Numbers followed by the same letter do not differ at the 5% level by Duncan's new multiple range test.

Table III. *Specific Activities of $N_2[C_2H_2]$ Fixation in Ambient pO_2 following Long Term Exposure to Subambient pO_2s*

pO_2	$N_2[C_2H_2]$ Fixation: Exposed to Indicated pO_2 for 95 hr	$N_2[C_2H_2]$ Fixation: Reincubated in 0.21-atm O_2 after 99 hr
atm	μg $N_2[C_2H_2]$ fixed/g nodule fresh wt·hr	
0.02	65.2 c*	19.1 d
0.06	137.2 a	75.0 bc
0.21	113.1 ab	129.1 a

*Numbers followed by the same letter do not differ at the 5% level by Duncan's new multiple range test.

system of intact soybean nodules to variant pO_2s is different from that previously reported in *Azotobacter* where both respiratory and conformational protection mechanisms are postulated (18). An impressive feature of one of the O_2 protection responses in *Azotobacter* is the rapidity with which the N_2 fixation system can either reversibly "switch on" or "switch off" within minutes to protect nitrogenase. Nitrogen fixation activities of intact soybeans were decreased rapidly following exposure to variant pO_2s within a wide range (0.02–0.32 atm O_2), but unlike *Azotobacter*, control nitrogenase activities were re-established after a considerable period of time of exposure to a variant pO_2. Upon re-exposure of intact plant nodules to ambient O_2 conditions, $N_2[C_2H_2]$ fixation rates were again rapidly repressed, and it appeared that an equivalent lengthy time period was required to re-establish control rates.

There are many possible factors that could be involved in the adaptive response of intact plants to variant pO_2s. The response may involve changes in cortical resistance to O_2 diffusion, alterations in vesicle membrane resistance to O_2 transport, changes in leghemoglobin forms (10) or content, conformational changes in nitrogenase, respiratory changes, translocation alterations, or changes in Cyt P-450 which has been implicated in the O_2 transport scheme (1). Presently, the physiological nature of the adaptive response is an enigma; however, one interpretation of the data is that nodules of intact soybean plants exposed to a variant pO_2 have mechanisms that permit the maintenance of an optimal internal pO_2 for ATP production. This capability probably has agronomic significance in soybean production situations where the soil pO_2 is reduced by conditions such as flooding or soil compaction.

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LITERATURE CITED

1. Appleby, C. A., G. L. Turner, and P. K. Macnicol. 1975. Involvement of oxyleghaemoglobin and cytochrome P-450 in an efficient oxidative phosphorylation pathway which supports nitrogen fixation in *Rhizobium*. Biochim. Biophys. Acta 387: 461–474.
2. Bergersen, F. J. 1962. Oxygenation of leghemoglobin in soybean root nodules in relation to the external oxygen tension. Nature 194: 1059–1061.
3. Bergersen, F. J. 1970. The quantitative relationship between nitrogen fixation and the acetylene-reduction assay. Aust. J. Biol. Sci. 23: 1015–1025.
4. Bergersen, F. J. 1971. Biochemistry of symbiotic nitrogen fixation in legumes. Annu. Rev. Plant Physiol. 22: 121–140.
5. Bergersen, F. J. and D. J. Goodchild. 1973. Aeration pathways in soybean root nodules. Aust. J. Biol. Sci. 26: 729–740.
6. Bergersen, F. J. and G. L. Turner. 1975. Leghaemoglobin and the supply of O_2 to nitrogen fixing root nodule bacteroids: studies of an experimental system with no gas phase. J. Gen. Microbiol. 89: 31–47.
7. Burris, R. H., W. E. Magee, and M. K. Bach. 1955. The pN_2 and the pO_2 function for nitrogen fixation by excised soybean nodules. Ann. Acad. Sci. Fenn. 60: 190–199.
8. Criswell, J. G., R. W. F. Hardy, and U. D. Havelka. 1976. Nitrogen fixation in soybeans: measurement techniques and examples of applications. *In:* L. D. Hill, ed., Proc. of the World Soybean Res. Conf., University of Illinois, Urbana-Champaign, 1975. The Interstate Printers & Publishers, Inc., Danville, Ill. pp. 108–124.
9. Criswell, J. G., U. D. Havelka, B. Quebedeaux, and R. W. F. Hardy. 1975. Nitrogen fixation under altered rhizosphere pO_2 by excised and intact plants of soybeans. Agron. Abstr. p. 131.
10. Fuchsman, W. H., C. R. Barton, M. M. Stein, J. T. Thompson, and R. M. Willett. 1976. Leghemoglobin: different roles for different components? Biochem. Biophys. Res. Commun. 68: 387–392.
11. Hardy, R. W. F., R. C. Burns, R. R. Hebert, R. D. Holsten, and E. K. Jackson. 1971. Biological nitrogen fixation: a key to world protein. Plant Soil Special Vol.: 561–590.
12. Hardy, R. W. F., R. C. Burns, and R. D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5: 47–81.
13. Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43: 1185–1207.
14. Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. No. 347.
15. Karlsson, B. 1966. Heat treatment of molecular sieves for direct separation of argon and oxygen at room temperature by gas chromatography. Anal. Chem. 38: 668–669.
16. Moorby, J., M. Ebert, and N. T. S. Evans. 1963. The translocation of ^{11}C-labelled photosynthate in the soybean. J. Exp. Bot. 14: 210–220.
17. Pearson, R. W. 1965. Soil environment and root development. *In:* W. H. Pierre, D. Kirkham, J. Pesek, and R. Shaw, eds., Plant Environment and Efficient Water Use. American Society of Agronomy and Soil Science Society of America, Madison, Wisc. pp. 95–126.
18. Postgate, J. 1971. Biochemical and physiological studies with free-living, nitrogen-fixing bacteria. Plant Soil. Special Vol.: 551–559.
19. Quebedeaux, B., U. D. Havelka, K. L. Livak, and R. W. F. Hardy. 1975. Effect of altered pO_2 in the aerial part of soybean on symbiotic N_2 fixation. Plant Physiol. 56: 761–764.
20. Steel, G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. p. 114.
21. Stolzy, L. H. and j. letey. 1964. Characterizing soil oxygen conditions with a platinum microelectrode. Adv. Agron. 16: 249–279.
22. Tjepkema, J. D. and C. S. Yocum. 1974. Measurements of oxygen partial pressure within soybean nodules by oxygen microelectrodes. Planta 119: 351–360.
23. Wittenberg, J. B., C. A. Appleby, F. J. Bergersen, and G. L. Turner. 1975. Leghemoglobin: the role of hemoglobin in the nitrogen-fixing legume root nodule. Ann. N. Y. Acad. Sci. 244: 28–34.