Response to Drought Stress of Nitrogen Fixation (Acetylene Reduction) Rates by Field-Grown Soybeans

P. RANDALL WEISZ, ROBERT F. DENISON, AND THOMAS R. SINCLAIR*
Agronomy Department and United States Department of Agriculture, Agricultural Research Service, Agronomy Physiology Laboratory, University of Florida, Gainesville, Florida 32611

ABSTRACT

The effects of drought stress on soybean nodule conductance and the maximum rate of acetylene reduction were studied with *in situ* experiments performed during two seasons and under differing field conditions. In both years drought resulted in decreased nodule conductances which could be detected as early as three days after water was withheld. The maximum rate of acetylene reduction was also decreased by drought and was highly correlated with nodule conductance ($r = 0.95$). Since nodule conductance is equal to the nodule surface area times the permeability, the relationship of these variables to both whole-plant and unit-nodule nitrogenase activity was explored. Drought stress resulted in a decrease in nodule gas permeability followed by decreases in nodule surface area when drought was prolonged. Under all conditions studied acetylene reduction on a unit-nodule surface area basis was highly correlated with nodule gas permeability ($r = 0.92$). A short-term oxygen enrichment study demonstrated nodule gas permeability may limit oxygen flux into both drought-stressed and well-watered nodules of these field-grown soybeans.

Drought stress decreases nitrogen fixation rates as measured by both acetylene reduction (1, 7, 17-22) and ^{15}N uptake (16). While some researchers have concluded that this loss of nodule activity is due to an inhibition of photosynthesis (8, 9, 13), Sprent (22) concluded that nitrogen fixation is more sensitive than photosynthesis to drought stress. This is supported by the findings of Bennett and Albrecht (1) who found that nitrogen fixation was closely correlated with nodule water potential which, in turn, was more sensitive to drought stress than was either leaf water potential or diffusive conductance. Pankhurst and Sprent (11, 12) postulated that the primary effect of drought stress on nitrogen fixation was to depress oxygen uptake and therefore respiration, causing a depletion of ATP inside the nodule. They presumed that the depressed oxygen uptake was caused by either a loss of oxygen conductance through the nodule or by inhibition of oxygen requiring reactions, or perhaps by both. Restricted oxygen conductance has been shown by theoretical arguments to be an important feature of viable, well-watered nodules (16).

With the development of an *in situ* system to make measurements of acetylene reduction rates under field conditions (3) and an analysis scheme to calculate the maximum rate of acetylene reduction, the nodule gas conductance, and the Michaelis-Menten constant (4), quantitative observations of the potential limitation of nodule conductance on acetylene reduction rates could be made. Further, conductance could be broken into its two components, permeability and surface area,

$$k = P*A, \qquad (1)$$

where k = nodule conductance (mm^3/s), P = the mean nodule gas permeability of all the nodules in the root chamber (mm/s), and A = total nodule surface area in the root chamber (mm^2).

If the nodule gas permeability was found to influence acetylene reduction rates, this would indicate that O_2 diffusion into nodules might be an important factor in nitrogen fixation rates. On the other hand, if the nodule surface area was the main parameter which determined k, then O_2 diffusion might not be an important factor influencing nitrogen fixation.

A more direct test of the potential limitation of nodule permeability to O_2 diffusion would be to subject nodules to atmospheres of elevated O_2 concentrations. If the initial effects of drought stress on nitrogen fixation are largely due to a decreased O_2 permeability through the nodule cortex, elevated O_2 concentrations in the atmosphere surrounding drought-stressed or dessicated nodules should result in a recovery of acetylene reduction rates. Such observations have been reported (12) for detached drought-stressed nodules. Ralston and Imsande (14) reported that the loss of nodule activity associated with nodule excision could be overcome by exposure to elevated concentrations of O_2. They suggested this was due to a decreased nodule O_2 permeability associated with nodule dessication. The effects of elevated partial pressure of O_2 on drought-stressed nodules have not been reported for *in situ* field conditions.

The purpose of this research was 2-fold. First, to determine the effects of drought stress on nodule conductance, nodule gas permeability, and nodule surface area. Second, to study the significance of each of these parameters in determining whole plant acetylene reduction rates. An experiment using elevated partial pressures of O_2 was also performed to determine if nodule gas permeability might be a limiting factor for acetylene reduction rates under well-watered and drought-stressed field conditions. Finally, a relationship between whole-plant acetylene reduction and nodule conductance, and between unit-nodule acetylene reduction and nodule gas permeability is proposed.

MATERIALS AND METHODS

In Situ Measurements. The acetylene reduction rates of nodulated soybean (*Glycine max*. [L.] Merr.) were measured *in situ* under field conditions using the system described previously (3). Briefly, at the time of sowing, open-ended chambers were installed in crop rows that had been previously machine seeded. Acetylene reduction chambers, 16 cm long and 10 cm in diameter, were filled with field soil and three seeds sown at a 2-cm depth. Upon emergence of the second trifoliolate leaf, plants were thinned to one per chamber. When acetylene reduction rates were to be measured, a gas tight lid was placed on the chamber with a seal around the base of the plant stem. A low concentration acetylene-air mixture (0.08 $nmol/mm^3$ in 1980, 0.04 $nmol/mm^3$ in 1982) was continuously flowed into the bottom of the chamber at 17 cm^3/s. A slight vacuum drew the

acetylene-air mixture through the chamber and back to a mobile laboratory at 1.7 cm^3/s, where the concentration of ethylene and acetylene in the gas mixture was determined with a gas chromatograph fitted with a flame ionization detector. The gas flow from each chamber was analyzed every half hour.

1980 Field Experiment. A preliminary study on the effects of drought stress was conducted in 1980 by analyzing data obtained during a naturally occurring drought stress. The cultivar Chippewa 64 was grown in Ithaca, NY. Field preparation and plant materials have been described previously (2). The unique feature for this study was that there was only trace rainfall after August 15 through August 30, while the evaporative demand remained high. Data for the period of August 21 to August 28 were analyzed for changes in nodule gas conductance and maximum acetylene reduction velocity associated with the drought period by the approach of Denison *et al.* (4). This was done each night by flowing three additional concentrations of acetylene (0.04, 0.20, and 0.40 nmol/mm^3) through the chambers. The ethylene production rate data from the four acetylene concentrations were used to solve for nodule conductance, maximum velocity of acetylene reduction, and the Michaelis-Menten constant for the plant nitrogenase system.

1982 Field Experiments. Four drought cycles were performed in Gainesville, FL in 1982. Field preparation, plant materials, and the modified method used for calculating the maximum acetylene reduction rate and nodule conductance used in 1982 are described in detail elsewhere (2, 5). This study was done with the soybean cultivar Biloxi which was seeded in the field and chambers on March 23. This cultivar is an indeterminate line which, when seeded early in the spring, has a long vegetative period. By the end of June when the experiments were terminated, these plants had not yet produced flowers and had approximately 20 nodes on the main stem. Each of the four experiments was initiated by irrigating all the test chambers with 125 ml water, and then sealing a lid on each of the chambers. For the well-watered treatments, an additional 125 ml was added to the top of the chambers at 2- to 3-d intervals. The drought stress chambers were left unwatered and allowed to dry down over the course of the experiment. The soil at this location (Arrendondo fine sand) had a limited water-holding capacity so that the plants were usually visibly wilted in 8 to 10 d if there was no precipitation.

The number of plants and duration of each of the drought cycles varied. The first cycle from April 30 to May 9 when the plants had approximately five leaves, had seven plants in each treatment. The second cycle was from May 10 to May 16 when there were approximately eight leaves on the seven plants per treatment. The third cycle was from May 17 to May 22 when the plants had nine leaves and there were seven plants per treatment. The fourth cycle was when the plants had 13 leaves, and was from June 2 to June 10. In this cycle all 14 plants were not watered for the first 5 d, and then on the 6th and 7th d seven plants received 125 ml water each. On each day of a drought cycle a two-tailed *t*-test ($\alpha = 0.05$) was used to determine if differences existed between the well-watered and the drought-stressed treatments for either the maximum rate of acetylene reduction or nodule conductance. At the end of each drought cycle all plants were harvested and plant height, leaf area, nodule number, and nodule fresh and dry weight were measured. A two-tailed *t*-test was used to determine if differences in these variables existed between the well-watered and the drought-stressed treatments.

Nodule Surface Area. Before drying the nodules after each harvest in 1982, the surface area of the nodules was determined. This procedure involved making a photocopy of all the nodules harvested from the root chamber for each plant. At a later date the largest diameter (*d*, mm) of each nodule was measured from

the photocopy, and the surface area (*A*, mm^2) determined from the following empirical equation:

$$A = (1.46 \times d^2) - 1.44 \qquad (2)$$

Equation 2 was developed from a detailed study of 100 nodules collected independently of the drought study. For each of these 100 nodules the length of each of the three axes was measured to the nearest 0.25 mm with the aid of a dissecting microscope. Also, the thickness of the nodule cortex in two axes was measured. The mean cortical thickness for the nodule was subtracted from the length of its three axes so that only the dimensions of the bacteroid-containing tissue was considered. As a result, the nodule surface areas reported here represent the surface area of the acetylene reducing tissue only. The dimensions of all three axes were significantly different so the surface area of the nodule was determined by using the general equation for the surface area of a quadratic surface (6). Equation 2 was obtained by regressing the tabulated surface area against the length of the longest axis for each of the 100 nodules studied ($r^2 = 0.95$).

Permeability Calculation. The data on nodule surface area were then used to calculate permeability from equation 1. Also, the saturated acetylene reduction rate on a whole plant basis (V_{max}, nmol C$_2$H$_2$ x plant^{-1} $\times$ s^{-1}) could be converted to express the rate per unit nodule surface area (*J*, nmol C$_2$H$_2$ $\times$ mm^{-2} $\times$ s^{-1}).

$$J = V_{max}/A \qquad (3)$$

Since the total nodule surface area might not have been constant over the course of an experiment, calculations of the maximum flux density of acetylene and the nodule permeability were only made based on the V_{max} and *k* obtained during the night prior to plant harvest. A two-tailed *t*-test ($\alpha = 0.05$) was used to determine if differences existed between the well-watered or drought-stressed treatments for either nodule surface area, maximum flux density of acetylene, or nodule gas permeability.

Oxygen Enrichment Experiments. Oxygen enrichment experiments were conducted on three nights during the 1982 season. Nodule conductance was determined just prior to altering the pO$_2$ in the assay chamber. After conductance was determined, the acetylene concentration in the chambers was returned to 0.04 nmol/mm^3 (1 matm) and the rate of acetylene reduction was measured approximately every 10 min. The rate of maximum acetylene reduction was calculated for each of these 10-min intervals using the analytical method described by Denison and Sinclair (2). This method assumes that the nodule conductance remained constant throughout the observation period, usually less than 5 h. The evening hours around sunset were chosen for this study because the soil temperature was stable. Typically, three estimates of maximum acetylene reduction at ambient pO$_2$ were made for each plant. Supplemental O$_2$ was then added to the air-acetylene mixture flowed to each root chamber. After about 55 min, the rates of acetylene reduction reached steady state and three estimates of maximum acetylene reduction were again made for each plant. Values of maximum flux density of acetylene at each pO$_2$ were calculated by dividing the maximum rate per plant by the nodule surface area.

On May 21, six well-watered and six drought-stressed plants were assayed at ambient and 0.4 atm pO$_2$. On June 9, five drought-stressed plants and four plants which had been rewatered that same day after 5 d of drought stress, were assayed at ambient, 0.4, and 0.6 atm pO$_2$. On June 11, four drought-stressed and five severely drought-stressed plants were assayed at ambient, 0.35, 0.45, and 0.55 atm pO$_2$. The pO$_2$ was calculated based on the flow rate of supplemental O$_2$ added to the total gas flow supplied to each acetylene reduction chamber.

RESULTS

In 1980, the conductance and maximum velocity of acetylene reduction declined in parallel continuously through the period of drought stress. When the maximum velocity of acetylene reduction was plotted against nodule conductance, it was found that the ratio of these parameters remained fairly stable through the drought period even though the magnitude of each of these variables was changed considerably. This is illustrated for three plants in Figure 1.

In 1982, the response of the plants during the first 5 to 6 d was similar for all the drought cycles. The maximum acetylene reduction rates and nodule conductivities decreased steadily for the droughted plants compared with the well-watered plants (Fig. 2, A and B). Statistical differences ($\alpha = 0.05$) could be demonstrated by day 5 or 6 of the treatment. No statistically significant differences in the nodule or plant morphological traits were found between the well-watered and drought-stress plants after either the 5- or 6-d drought cycles.

Extension of the drought treatment to 9 d as done for the April 30 to May 9 cycle resulted in further declines in maximum acetylene reduction activity and nodule conductance (Fig. 3, A and B). Statistical differences ($\alpha = 0.05$) were maintained for days 6 through 9. At harvest the drought stress treatment had statistically ($\alpha = 0.05$) less nodule fresh weight than the well-watered plants (0.60 g/plant versus 0.90 g/plant). No other differences in the plant or nodule traits studied were found between treatments.

Rewatering seven of the plants in the June 2 to June 10 cycle after a 5-d drought period was an attempt to explore the possibilities for nodule recovery from desiccation. As in the earlier cycles, maximum acetylene reduction and nodule conductance
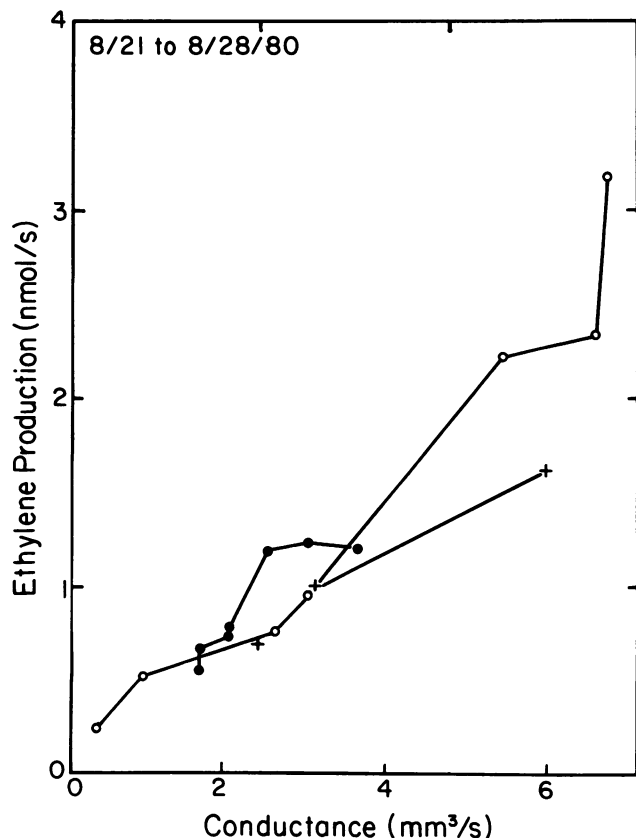

FIG. 1. Decline in maximum acetylene reduction and nodule conductance over a 7-d period during a drought in 1980. Data are for three plants.
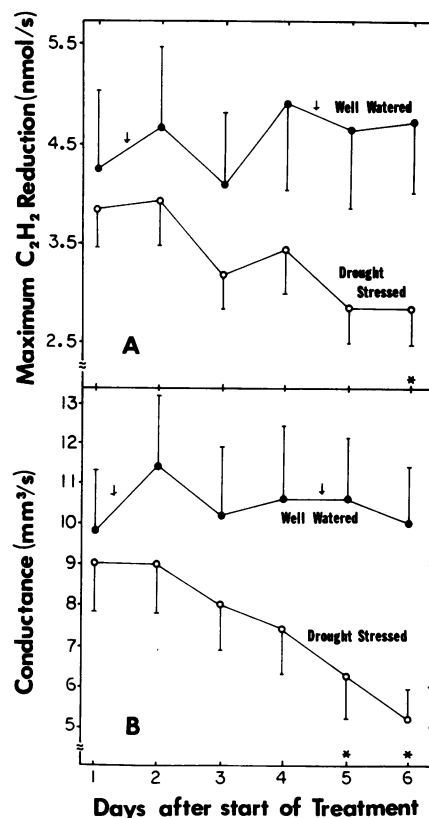

FIG. 2. A, Maximum acetylene reduction versus days after start of treatment; B, nodule conductance versus days after start of treatment. Arrows, supplementa. watering dates for well-watered treatment; *, means differ statistically ($\alpha = 0.05$). Error bars represent 1 SE.

decreased during the initial 5 d of drought stress, and continued to decrease in the plants which were not rewatered. Plants rewatered after 5 d of drought stress appeared to begin a recovery. After watering on day 6 nodule conductance increased, and on day 7 conductance differed statistically between the rewatered and drought-stressed plants ($\alpha = 0.10$). However, on day 8 values of maximum acetylene reduction and conductance for the rewatered plants were still lower than those observed on day 4. Upon harvesting these plants, none of the nodule or plant traits measured were found to differ between the two treatments.

There was a high correlation between maximum acetylene reduction and nodule conductance for all plants regardless of treatment. For drought-stressed plants the correlation coefficient (r) between these parameters was 0.95. For well-watered plants, nodule conductance and maximum acetylene reduction were similarly correlation ($r = 0.98$). Regression analysis indicated that the ratio of maximum acetylene reduction to nodule conductance was similar in both treatments. For these reasons, all the 1982 data were pooled and are presented in Figure 4. The overall correlation coefficient between maximum acetylene reduction and nodule conductance was 0.97.

Nodule gas permeability also decreased significantly as a consequence of drought stress in each of the experimental cycles. Data from the 5-, 6-, and 9-d drought cycles are presented in Table I. In contrast to the decline in permeability, nodule surface area (Table I) was not significantly affected by drought stress in either the 5- or 6-d study. However, prolonged exposure to drought stress did result in decreases in nodule surface area as evidenced in the 9-d study. Maximum flux density of acetylene was also decreased by exposure to drought stress in each experiment (Table I).
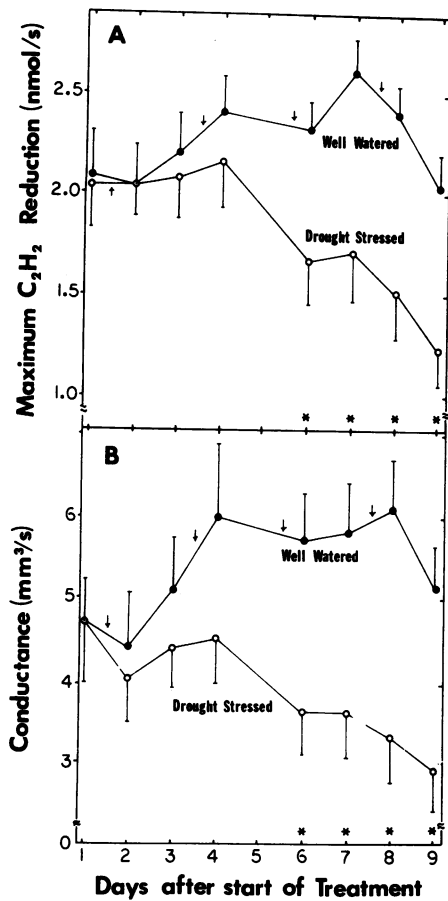
Maximum acetylene reduction was plotted against nodule

FIG. 3. A, Maximum acetylene reduction *versus* days after start of treatment; B, nodule conductance *versus* days after start of treatment. Arrows, supplemental watering dates for well-watered treatment; *, means differ statistically ($\alpha = 0.05$). Error bars represent 1 SE.
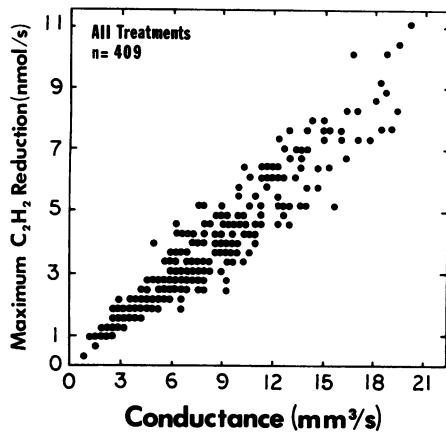

FIG. 4. Maximum acetylene reduction *versus* nodule conductance for all plants studied in 1982.

Table I. *Nodule Gas Permeability, Nodule Surface Area, and Acetylene Flux Density at the End of Each Drought Experiment*

	Experiment Duration (d)		
	5	6	9
	Gas Permeability[a] $mm\ s^{-1} \times 10^3$		
Well-watered	5.78 ± 0.26	7.04 ± 0.57	6.84 ± 0.69
Drought-stressed	3.61 ± 0.56	4.29 ± 0.34	5.04 ± 0.50
Level of significance	0.004	0.002	0.054
	Surface Area[a] mm^2		
Well-watered	1760 ± 275	1460 ± 254	785 ± 253
Drought-stressed	1660 ± 221	1200 ± 154	556 ± 152
Level of significance	0.790	0.490	0.069
	C_2H_2 Flux Density[a] $nmol\ mm^{-2}\ s^{-1} \times 10^3$		
Well-watered	2.83 ± 0.22	2.90 ± 0.27	2.76 ± 0.31
Drought-stressed	1.89 ± 0.32	2.23 ± 0.10	2.18 ± 0.20
Level of significance	0.033	0.012	0.140

[a] Mean ± SE.

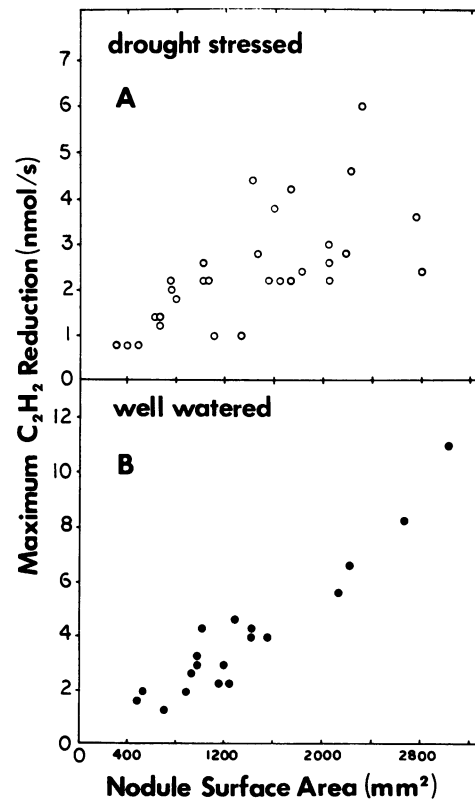**FIG. 5.** Maximum acetylene reduction *versus* nodule surface area for (A) drought-stressed plants and (B) well-watered plants.

surface area for drought-stressed and well-watered plants in Figure 5, A and B, respectively. For drought-stressed plants the correlation between maximum acetylene reduction and nodule surface area was poor ($r = 0.66$). However, for well-watered plants the correlation was considerably better ($r = 0.94$). Maximum acetylene reduction was plotted against nodule gas permeability in Figure 6. Within either the drought-stressed or the well-watered treatment a significant ($\alpha = 0.05$) correlation between these variables could not be demonstrated. When the data were pooled, there was only a weak correlation ($r = 0.31$). In contrast

to this poor correlation between nodule gas permeability and whole plant acetylene reduction, the flux density of acetylene and nodule gas permeability were highly correlated for all plants studied ($r = 0.92$, Fig. 7).

Increased pO_2 heightened the maximum flux density of acetylene reduction. However, the greater the degree of drought stress, the less the response to increased pO_2. Results from well-
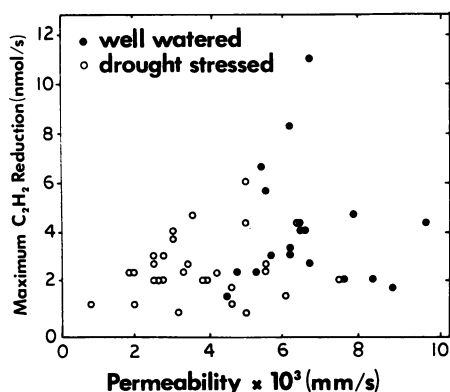
FIG. 6. Maximum acetylene reduction *versus* nodule gas permeability for drought-stressed and well-watered plants.
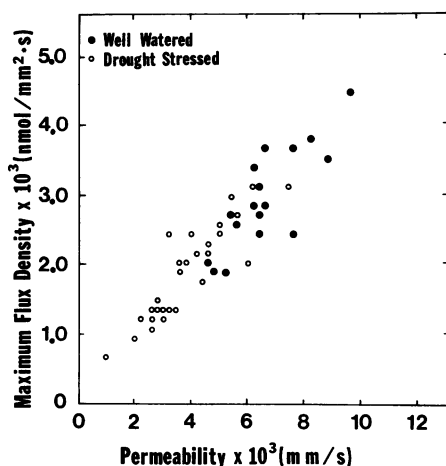

FIG. 7. Maximum flux density of acetylene *versus* nodule gas permeability for well-watered and drought-stressed plants.
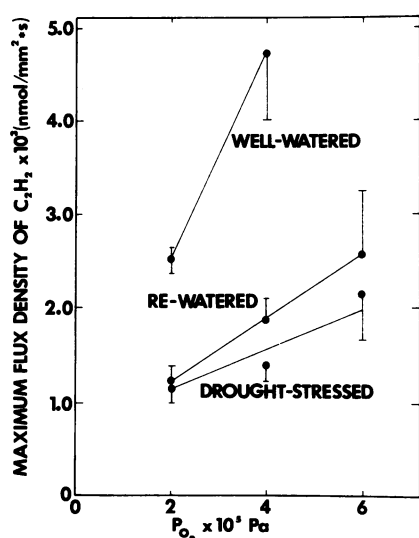

FIG. 8. Mean maximum flux density of acetylene *versus* partial pressure of O_2 for three treatment groups, well-watered, drought-stressed, and a group of re-watered after 5-d of drought stress. Bars are SE.

watered plants assayed on May 21, and two drought-stressed treatments assayed on June 9, are presented in Figure 8. Well-watered plants consistently responded more dramatically to increased pO_2 than did drought-stressed plants. At elevated pO_2,

maximum flux density of acetylene for drought-stressed plants returned to levels associated with well-watered plants.

DISCUSSION

The data obtained in both 1980 and 1982, under differing soil and climatic conditions, showed that drought stress resulted in decreases in both maximum acetylene reduction rate and nodule gas conductance. In 1982, this decrease was found as early as 3 d after water was withheld from the field plants (Fig. 3, A and B). After 5 and 6 d of drought stress, no effect could be detected in leaf area, plant height, nodule number, or nodule fresh and dry weight. However, when the drought period was prolonged, as in the 9-d study, nodule fresh weight was shown to be affected by the exposure to stress. These data indicated that the early effects of drought stress appeared to be limited to a decrease in nodule conductance and maximum acetylene reduction rate, which was followed by a subsequent loss in nodule weight.

Analysis of the acetylene reduction data in terms of nodule gas permeability showed that in the 5- and 6-d drought studies, permeability was significantly decreased. This indicated that the initial effect of drought stress on nodule conductance was a decrease in the gas permeability of the nodules and this was accompanied by a decrease in the flux density of acetylene. Pankhurst and Sprent (11) observed that one of the initial nodule responses to drought stress was the collapse of the intercellular air spaces in the lenticels associated with the outer nodule cortex. They postulated that the closure of these airspaces resulted in a decrease in O_2 permeability through the nodule which caused a decrease in nitrogenase activity as ATP supplies were depleted. The decreases in nodule gas permeability reported here in association with drought stress give quantitative confirmation to their anatomical observations.

The results of the O_2 enrichment study with drought-stressed nodules are also in close agreement with those reported for detached nodules (12). Elevated levels of O_2 increased the maximum flux density of acetylene and returned the activity of drought-stressed nodules to those associated with well-watered conditions. These observations further suggest that the early effects of drought stress on nitrogenase activity involve an O_2 limitation.

Because nodule gas permeability appeared to play a role in determining acetylene reduction rates under drought-stressed conditions, nodule surface area alone was not a good predictor of whole-plant nitrogenase activity under these conditions (Fig. 5). However, under well-watered conditions, nodule surface area was well correlated with maximum acetylene reduction. Figure 7 indicates that under well-watered conditions a maximum nodule gas permeability may be achieved and variability in nodule conductance or total plant acetylene reduction is then associated with changes in nodule surface area per plant. As nodule surface area may be closely related to total nodule mass, it is not surprising that high correlations between nodule mass and total nodule activity have been reported in the literature (10). However, the role of nodule gas permeability in well-watered plants is of curiosity. Elevated levels of O_2 resulted in nearly double the flux density of acetylene in well-watered plants (Fig. 8). Seemingly the rate of acetylene reduction under well-watered field conditions was also sensitive to the supply of O_2 to the nitrogen fixing tissue. Similar findings have been reported for intact potted plants of white clover (15), red clover, lucerne, sainfoin, pea soybean, and other legumes (24).

In light of these findings, we suggest that a good model for understanding plant acetylene reduction rates is to determine flux per unit nodule surface area as a function of nodule permeability (Fig. 7). The nodule permeability appears to have a strong influence on the acetylene reduction rate per unit surface presumably due to the regulation of O_2 diffusion. A decreased permea-

bility would restrict further O_2 diffusion and account for the observed decrease in the acetylene reduction rate per unit surface area. Having accounted for the effect of permeability on rate per unit surface area, then the fixation for a plant can be determined by multiplying by total nodule surface area. The result is that total acetylene reduction rate per plant is closely associated with nodule conductance. Our findings with intact field-grown plants as well as the results reported by others (11, 12, 14, 15, 23) indicate that the diffusion of O_2 in nodules is an important factor in the regulation of nitrogen fixation rates.

LITERATURE CITED

1. BENNETT JM, SL ALBRECHT 1984 Drought and flooding effects on N_2 fixation, water relations, and diffusive resistance of soybean. Agron J 76: 735–740
2. DENISON RF, TR SINCLAIR 1985 Diurnal and seasonal variation in nitrogen fixation (acetylene reduction) rates by field-grown soybeans. Agron J. In press
3. DENISON RF, TR SINCLAIR, RW ZOBEL, MM JOHNSON, GM DRAKE 1983 A non-destructive field assay for soybean nitrogen fixation by acetylene reduction. Plant Soil 70: 173–182
4. DENISON RF, PR WEISZ, TR SINCLAIR 1983 Analysis of acetylene reduction rates of soybean nodules at low acetylene concentration. Plant Physiol 73: 648–651
5. DENISON RF, PR WEISZ, TR SINCLAIR 1985 Variability among plants in nitrogen fixation (acetylene reduction) rates by field-grown soybeans. Agron J. In press
6. ELLIS R, D GULLIC 1970 Calculus with Analytical Geometry. Harcourt Brace Jovanovich, Inc, New York
7. ENGIN M, JI SPRENT 1973 Effects of water stress on growth and nitrogen-fixing activity of *Trifolium repens*. New Phytol 72: 117–126
8. HUANG C, JS BOYER, LN VANDERHOEF 1975a Acetylene reduction (nitrogen fixation) and metabolic activities of soybean having various leaf and nodule water potentials. Plant Physiol 56: 222–227
9. HUANG C, JS BOYER, LN VANDERHOEF 1975b Limitation of acetylene reduc-

tion (nitrogen fixation) by photosynthesis in soybean having low water potentials. Plant Physiol 56: 228–232
10. LAWN RJ, WA BRUN 1974 Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. Crop Sci 14: 11–16
11. PANKHURST CE, JI SPRENT 1975a Surface features of soybean root nodules. Protoplasma 85: 85–98
12. PANKHURST CE, JI SPRENT 1975b Effects of water stress on the respiratory and nitrogen-fixing activity of soybean root nodules. J Exp Bot 26: 287–304
13. PATTERSON RP, CD RAPER, JR, HD GROSS 1979 Growth and specific nodule activity of soybean during application and recovery of a leaf moisture stress. Plant Physiol 64: 551–556
14. RALSTON EJ, J IMSANDE 1982 Entry of oxygen and nitrogen into intact soybean nodules. J Exp Bot 33: 208–214
15. SHEEHY JE, FR MINCHIN, JF WITTY 1983 Biological control of resistance to oxygen flux in nodules. Ann Bot 52: 565–571
16. SINCLAIR TR, J GOUDRIAAN 1981 Physical and morphological constraints on transport in nodules. Plant Physiol 67: 143–145
17. SPRENT JI 1971 The effects of water stress on nitrogen fixing root nodules. I. Effects on the physiology of detached soybean nodules. New Physiol 70: 9–17
18. SPRENT JI 1972a The effects of water stress on nitrogen-fixing root nodules. II. Effects on the fine structure of detached soybean nodules. New Phytol 71: 443–450
19. SPRENT JI 1972b The effects of water stress on nitrogen-fixing root nodules. III. Effects of osmotically applied stress. New Phytol 71: 451–460
20. SPRENT JI 1972c The effects of water stress on nitrogen-fixing root nodules. IV. Effects on whole plants of *Vicia faba* and *Glycine max*. New Phytol 71: 603–611
21. SPRENT JI 1975 Nitrogen fixation by legumes subjected to water and light stress. *In* PS Nutman, ed, Symbiotic Nitrogen Fixation in Plants. Cambridge University Press, Cambridge, England
22. SPRENT JI 1976 Water deficits and nitrogen-fixing root nodules. *In* TT Kozlowski, ed, Water Deficits and Plant Growth, Vol IV. Academic Press, New York
23. WINSHIP LJ, JD TJEPKEMA 1983 The role of diffusion in oxygen protection of nitrogenase in nodules of *Alnus rubra*. Can J Bot 61: 2930–2936
24. WITTY JF, FR MINCHIN, JE SHEEHY 1983 Carbon costs of nitrogenase activity in legume root nodules determined using acetylene and oxygen. J Exp Bot 34: 951–963