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Canopy and Seasonal Profiles of Nitrate Reductase in Soybeans (*Glycine max* L. Merr.)[1]

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ABSTRACT

Nitrate reductase activity of soybeans (*Glycine max* L. Merr.) was evaluated in soil plots and outdoor hydroponic gravel culture systems throughout the growing season. Nitrate reductase profiles within the plant canopy were also established. Mean activity per gram fresh weight per hour of the entire plant canopy was highest in the seedling stage while total activity (activity per gram fresh weight per hour times the total leaf weight) reached a maximum when plants were in the full bloom to midpod fill stage. Nitrate reductase activity per gram fresh weight per hour was highest in the uppermost leaf just prior to full expansion and declined with leaf positions lower in the canopy. Total nitrate reductase activity per leaf was also highest in the uppermost fully expanded leaf during early growth stages. Maximum total activity shifted to leaf positions lower in the plant canopy with later growth stages.

Nitrate reductase activity of soybeans grown in hydroponic systems was significantly higher than activity of adjacent soil grown plants at later growth stages, which suggested that under normal field conditions the potential for nitrate utilization may not be realized. Nitrate reductase activity per gram fresh weight per hour and nitrate content were positively correlated over the growing season with plants grown in either soil or solution culture. Computations based upon the nitrate reductase assay of plants grown in hydroponics indicated that from 1.7 to 1.8 grams N could have been supplied to the plant via the nitrate reductase process. The harvested seed contained 1.1 to 1.2 grams N per plant. Thus, based on previous estimates of approximately 32% of the final N distribution being in the vegetative plant parts, the estimated input of reduced nitrogen via the enzyme assay was in agreement with the actual N accumulation.

The amount of calculated N_2-fixation by nodules per season with plants grown in hydroponics was less than 2% of the computed nitrate reduced via leaf nitrate reductase. Thus, the level of nitrate in the nutrient solution appeared to be quite inhibitory to N_2-fixation.

The response of the soybean plant to nitrogen is confounded by the ability of the plant to utilize both nitrate and N_2. Nitrate is considered the primary source of nitrogen available from the soil. The uptake of nitrate and subsequent reduction by nitrate reductase is the primary pathway of soil nitrogen utilization. The utilization of N_2 through the symbiotic relationship with *Rhizobium japonicum* (Kirchner) Buchanan affords a second major pathway of nitrogen input to soybeans.

Nitrogen fixation is normally initiated in soybeans 20 to 30 days after planting (3). Thus, initial nitrogen requirements must be met through utilization of nitrogen from the seed and nitrogen from the soil. Recent estimates indicate that some 25 to 30% (80–110 kg N per hectare per season) of the total plant nitrogen was supplied through the N_2-fixation process in soybeans as measured with the acetylene-reduction technique (3, 4). It has also been estimated by Kjeldahl and ^{15}N analysis that 95 kg N per hectare per season were supplied through fixation by soybeans (11). Sloger (personal communication) estimates that upwards of 50% of the nitrogen of mature plants may be derived from fixation. Thus a considerable portion of nitrogen in soybeans must be derived from nitrate. Little information is available on the seasonal potential of soybeans to utilize nitrate via nitrate reductase.

The present study was initiated to assess the relative ability of the soybean to utilize nitrate and N_2. Seasonal patterns of nitrate utilization via nitrate reductase were established. The potential to fix N_2 over the growing season was assessed by the acetylene-reduction method (3).

MATERIALS AND METHODS

Soybeans (*Glycine max* L. Merr. var. Beeson) were inoculated and planted May 18, 1970 in outdoor hydroponic gravel culture beds and in adjacent soil plots. The plants in the gravel beds were germinated with 0.1 mM $Ca(NO_3)_2$ and nutrient treatments were initiated 2 weeks later on June 1 at which time the unifoliolate leaves were just fully expanded. Nutrient treatments were designated 1/4X and 1X, as described previously (5). Complete nutrient solution changes were made at weekly intervals.

Nutrient treatments and the adjacent soil plots were replicated four times. Eight gravel culture beds, described previously (5), were used for each nutrient treatment with two beds per replication. The gravel culture beds and soil plots were over planted and thinned following emergence to 20 plants

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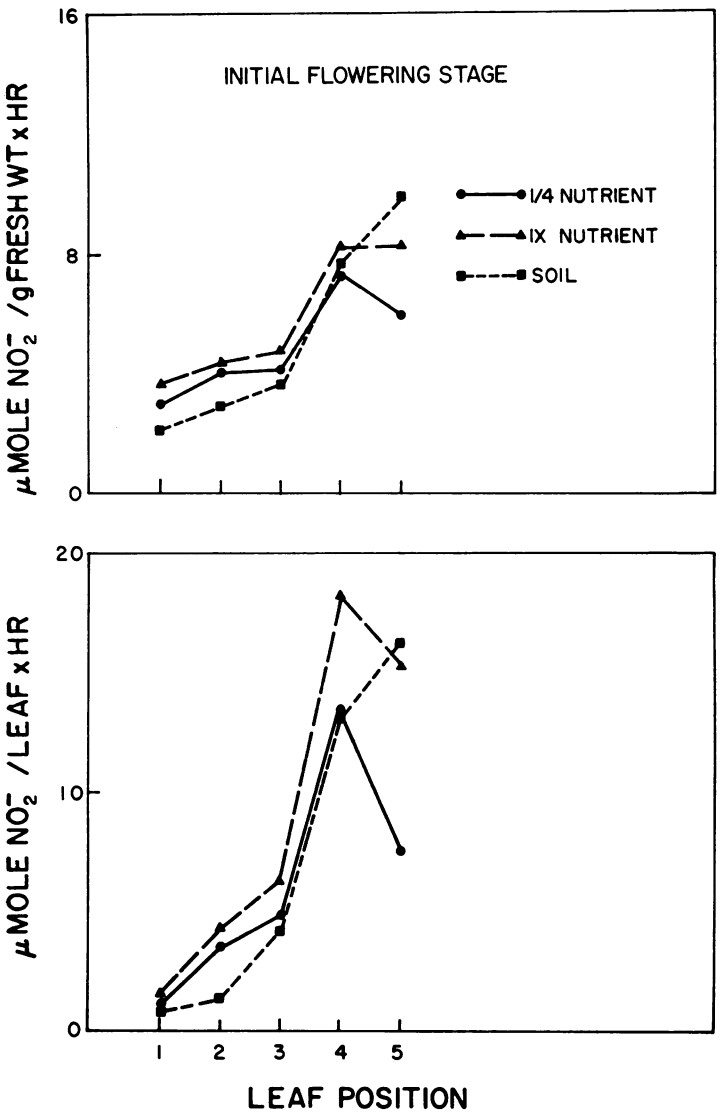

FIG. 1. Canopy profiles of nitrate reductase activity of soybeans 31 days after emergence (June 24). Leaves numbered consecutively from bottom to top of the plant with the unifoliolate leaf designated No. 1. Treatments are described in "Materials and Methods."

per meter. Mylar[2] sleeves, 10 cm in diameter and 25 cm long, were placed in the gravel beds to separate the roots of the individual plants. The sleeves were staggered in two rows with 10-cm intervals between sleeves in each row. One-half of the plants were planted in these sleeves for sampling, and the other half were planted between the sleeves and left to grow to maturity. At each sampling date, the sleeve and plant were removed as an intact unit and the gravel shaken from around the roots. The roots were severed from the tops and both were placed on ice until assayed.

Nitrate Reductase Assay. Plants were sampled at weekly intervals for determination of nitrate reductase activity of leaves from plants in both soil and hydroponic plots. The first two sampling dates involved nitrate reductase assays of unifoliolate leaves only. Thereafter, each trifoliolate leaf was assayed individually. Branches were removed as they developed to confine sampling to the trifoliolates on the main stem. Four plants were composited for each replication of the nutrient treatments and the soil plots. Nitrate reductase activity of leaves at each individual node was determined by a modification of the *in vivo* method of Mulder *et al.* (8) as modified by Klepper *et al.* (6).

A leaf disc punch was made by modifying a staple puller and mounting a 1 cm diameter copper cutter on one lip and a rubber stopper on the other lip to meet the cutter edge. With this leaf punch it was possible to punch 12 leaves simultaneously. Twenty-four leaf discs (from 12 leaflets) were obtained, weighed (approximately 0.2 g), and transferred to test tubes. Five milliliters of incubation media (0.2 M KNO_3 in 10 mM potassium phosphate, pH 7.5) were added to each tube. A stainless steel wire screen (20 mesh) was placed in each tube to hold the leaf discs below the solution surface. The samples were then evacuated for 2 min in a vacuum desiccator. Air was rapidly reintroduced and the procedure repeated. Samples were then transferred to a shaking water bath and incubated 1 hr at 30 C in the dark. After incubation the solution was poured off and 0.2-ml aliquots were analyzed for nitrite as described by Klepper *et al.* (6). Nitrate reductase

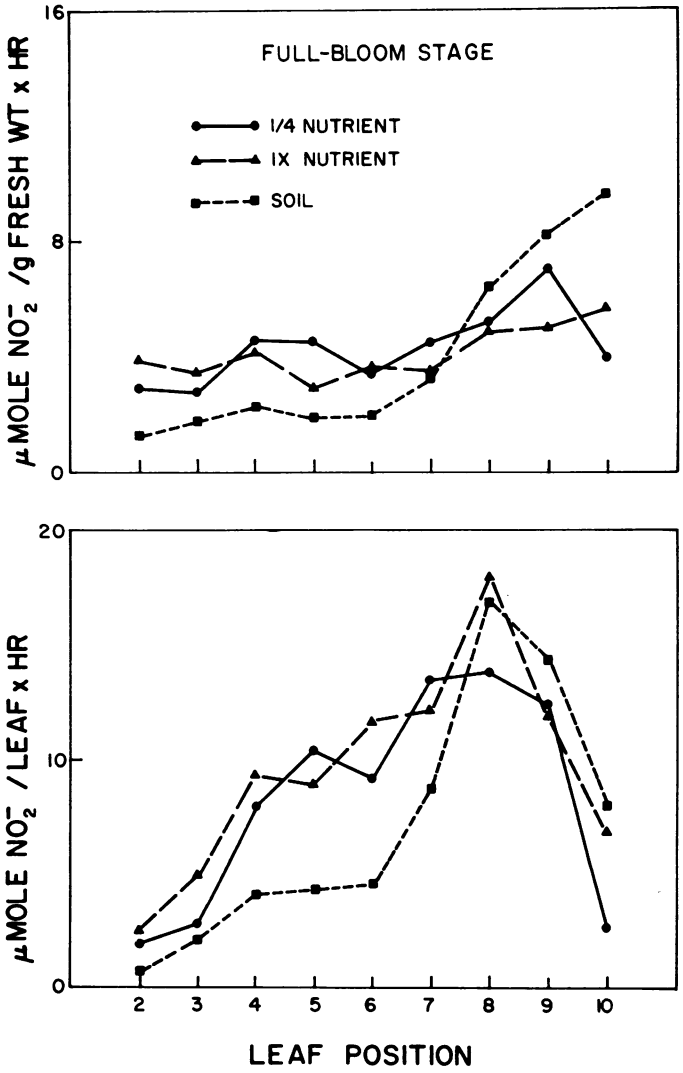

FIG. 2. Canopy profiles of nitrate reductase activity of soybeans 45 days after emergence (July 8). Leaf designation and treatments are as in Figure 1.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

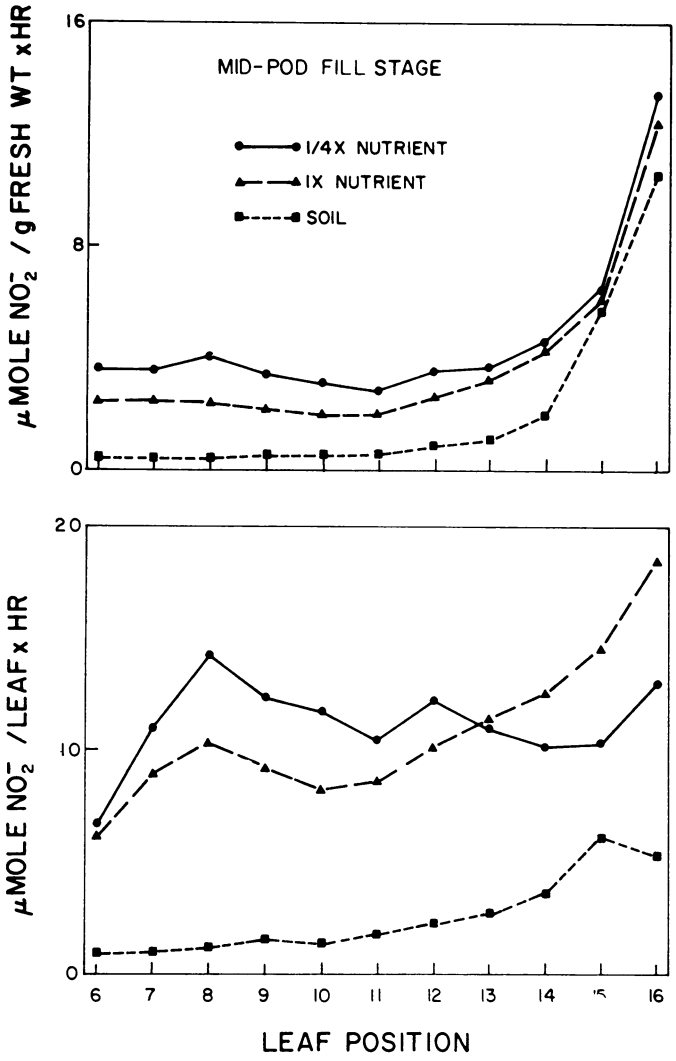

FIG. 3. Canopy profiles of nitrate reductase activity of soybeans 66 days after emergence (July 29). Leaf designation and treatments are as in Figure 1.

activity was expressed as μmoles NO_2^- formed per g fresh weight per hr. Activity per leaf was computed by multiplying activity per g fresh weight per hr by total fresh leaf weight. Activity per plant per day was estimated by summing the activities of the leaves at each node per hour $\times$ 24 $\times$ 0.8 (a correction factor for diurnal variation, see Fig. 7). Activity per plant per season was estimated by integrating the area under the curve obtained by plotting the activity per plant per day across the growing season.

Growth Stages. Growth stages (2) at which nitrate reductase canopy profile data are presented were as follows: "initial bloom"—five to six trifoliolate leaves unrolled and first flowers visible; "full bloom"—9 to 10 trifoliolate leaves unrolled and plants blooming at all nodes; "midpod fill"—pods plainly visible at top of plants and lower pods full length with beans beginning to form; "green bean"—bottom leaves beginning to "yellow" and top pods fully developed with beans approaching the "green bean" stage.

Nitrate Analysis. The remaining leaf material after removal of leaf discs was weighed and dried at 70 C. The dried plant tissue was extracted with hot water and nitrate determinations were made by the Technicon autoanalyzer method of Litchfield (7).

Nitrogenase Assay for N_2 Fixation Estimate. Fixation of N_2 by nodules from hydroponic grown plants was determined as described by Hardy *et al.* (4). Root segments exhibiting nodules were composited from two plants for a sample. Eight samples were taken for each nutrient treatment. The samples were incubated 1 hr at 30 C in the acetylene-argon-O_2 mixture. Following incubation, ethylene produced was determined by

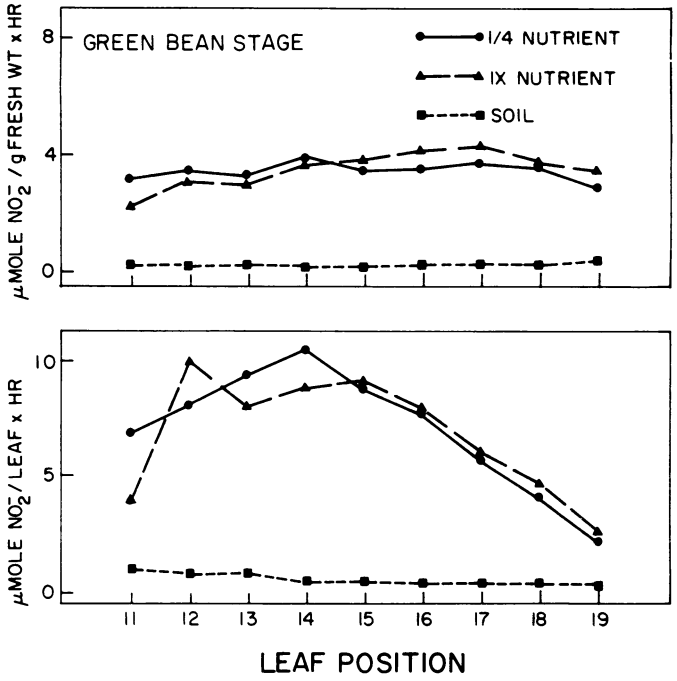

FIG. 4. Canopy profiles of nitrate reductase activity of soybeans 94 days after emergence (August 26). Leaf designation and treatments are as in Figure 1.

Table I. *Single Degree of Freedom Orthogonal Regression Analysis of Variation of Nitrate Reductase Canopy Profile Activity of Soybeans at Selected Growth Stages*

The growth stages initial bloom, full bloom, midpod fill, and green bean stages correspond with Figures 1 through 4, respectively.

Sampling Stage	Regression Component	Nitrate Reductase Activity per g fresh wt per hr		Nitrate Reductase Activity per leaf per hr	
		1X Nutrient *vs.* 1/4X nutrient	1X Nutrient *vs.* soil	1X nutrient *vs.* 1/4X Nutrient	1X Nutrient *vs.* soil
		F test			
Initial bloom (June 24)	Linear	3.60	11.14[1]	33.13[1]	0.02
	Quadratic	2.71	3.00	4.18[2]	9.55[1]
Full bloom (July 8)	Linear	0.25	36.70[1]	0.47	4.23[2]
	Quadratic	3.95	3.85	0.66	6.23[2]
Midpod fill (July 29)	Linear	1.19	0.12	25.10[1]	7.37[1]
	Quadratic	0.01	0.01	14.58[1]	1.46
Green bean (August 26)	Linear	19.20[1]	15.90[1]	0.54	6.99[1]
	Quadratic	2.74	15.43[1]	0.57	23.96[1]

[1] Significant at the 0.01 level.
[2] Significant at the 0.05 level.

gas chromatography using a hydrogen-flame detector. Column and chromatographic conditions were as outlined by Sloger (10). Nodule weights were determined after completion of the assay.

Statistical Analysis. The experimental design was a randomized complete block design with four replications. Nitrate reductase activity (both μmoles NO_2^- per g fresh weight per hr and μmoles NO_2^- per leaf per hr) was subjected to analysis of variance tests within sampling dates. LSD values were computed when significant F tests (0.05 level) were obtained among treatments. Orthogonal comparisons in regression were applied to test if treatment differences existed in profiles of nitrate reductase activity among leaf positions within sampling dates. Specific comparisons of the 1X treatment to the 1/4X nutrient treatment, and the 1X nutrient treatment to the soil treatment, were made.

RESULTS AND DISCUSSION

Canopy Profiles of Nitrate Reductase. Although nitrate reductase assays were made at weekly intervals, only those profiles at selected growth stages are given in Figures 1, 2, 3, and 4, respectively. Regression analysis of canopy profile differences between treatments for the growth stages in Figures 1 through 4 are given in Table I.

At the initial flowering stage nitrate reductase activity per g fresh weight per hr was highest with upper leaves in the plant canopy and consistently decreased with leaf positions lower in the plant canopy with all treatments (Fig. 1). Nitrate reductase activity per leaf per hr reflected the activity per g fresh weight per hr at early sampling stages with the exception that the uppermost trifoliolate was lower in activity per leaf per hr than the trifoliolate at node four with both hydroponic treatments. The lower activity at node five reflects the small size of the newest trifoliolate at the time of sampling.

Plants at the full bloom stage had 10 node positions and the unifoliolate leaf had abscised (Fig. 2). Nitrate reductase activity per g fresh weight per hr was highest with upper leaves on the plant and generally declined with lower leaves in the canopy. Similar profiles of activity per g fresh weight per hr within the plant canopy were evident in plants receiving the

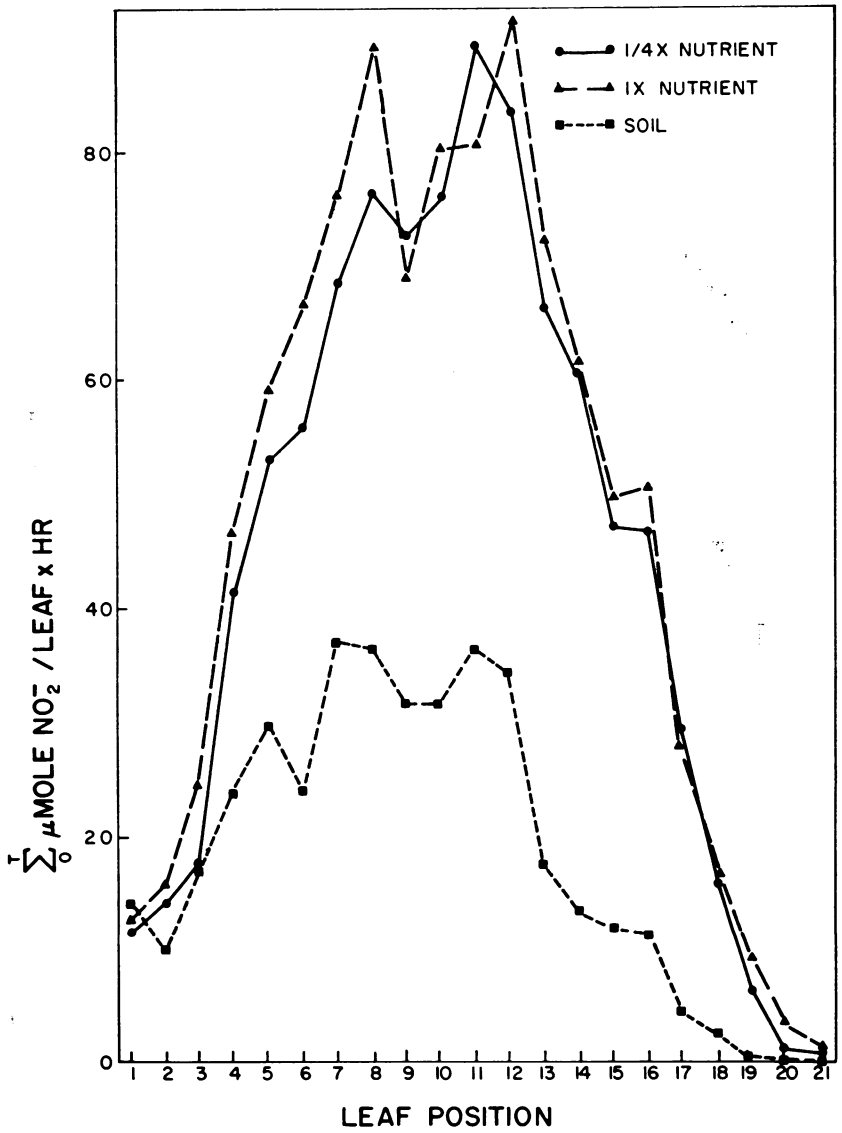

FIG. 5. Summation of nitrate reductase activity of individual leaves across all dates which the respective leaves were present on the plant. The unifoliolate leaf was designated No. 1 and the last trifoliolate to emerge was at the 21st node position. Treatments are described in "Materials and Methods."

different nutrient treatments. Analysis showed highly significant linear differences between nitrate reductase profiles of the 1X nutrient treatment and the soil treatment (Table I). Thus, distribution of nitrate reductase activity within the canopy was more uniform in plants grown in hydroponics than those grown in soil. Maximum nitrate reductase activity per leaf per hr at the full bloom stage had shifted to node positions further removed from the top of the plant (Fig. 2). With all treatments, activity peaked at node eight which was three positions from the top of the plant. Activity per leaf per hr was lowest with the bottom leaf remaining on the plant with all treatments. There was no difference between the activity profiles of plants in the nutrient treatments (Table I). Significant linear and quadratic components of the orthogonal comparison were found between profiles of nitrate reductase activity of the 1X nutrient treatment and the soil treatment. This indicated that the distribution of enzyme levels within the plant canopy was responding differently to soil and nutrient treatments.

At the midpod fill stage nitrate reductase activity per g fresh weight per hr was again highest with upper leaves on the plant, declined rapidly with the next two node positions, and was similar with the lower eight node positions within each treatment (Fig. 3). Profiles of activity per g fresh weight per hr were similar for all treatments. The profile of activity per leaf per hr revealed high activity with the upper nodes, reflecting the high specific activity, and a second peak becoming evident in hydroponic plants at node positions closer to the bottom of the plant. The second peak at node eight reflected the large leaf weight at that node. At the two subsequent weekly sampling dates (data not shown) maximum activity per g fresh weight per hr was found in the uppermost trifoliolate while total activity peaked some seven to eight nodes from the top of the plants. Thus, it was evident that in the intermediate growth stages activity per leaf per hr was maximal at node positions lower in the plant canopy.

At the green bean stage, nitrate reductase activity per g fresh weight per hr was relatively constant over the entire plant canopy (Fig. 4). Little difference in activity per g fresh weight per hr between the nutrient treatments was evident while activity of soil grown plants was consistently lower. Profiles of activity per leaf per hr reflected leaf size and peaked at leaf positions 12 and 14 for the 1X and 1/4X treatments, respectively (Fig. 4). Very little difference in the total activity profile was evident with soil grown plants.

It was apparent, when consideration was given to all the weekly profiles of nitrate reductase activity within the canopy, that the activity per g fresh weight per hr of the initially expanding leaf was low and did not reach maximal activity until almost fully expanded. After attaining maximum value the activity then slowly declined with age as additional new trifoliolates emerged. Although activity per g fresh weight per hr was maximal in upper leaves of the plant canopy at all sampling stages, activity per leaf per hr was more dependent upon leaf size, resulting in maximal activity at trifoliolate positions lower in the canopy.

Nitrate Reductase Seasonal Profiles. Summation of total potential nitrate reductase activity per leaf per hr for each leaf position over the season indicated maximum activity with leaves at the central nodes on the plant (Fig. 5). Peak activity was generally between leaf positions 8 and 12. Activity was lower with leaves 9 and 10; however, these trifoliolate leaves were badly wind damaged which likely accounted for the apparent lower levels of activity. Activity at the lower leaf positions was similar with soil and hydroponic grown plants while the activity of the third and subsequent leaf positions of the soil grown plants was markedly lower than the hydroponic grown plants. Although the levels of activity between soil and hydroponic grown plants was considerably different, both groups had peak activity with the centrally located trifoliolates. The peak activity with centrally located trifoliolates reflected both the size which these leaves attained and the duration of time which they were sampled.

Mean nitrate reductase activities per g fresh weight per hr and per leaf per hr and mean nitrate contents of the entire leaf canopy for the three treatments are shown in Table II.

Table II. *Mean Nitrate Reductase Activity and Mean Nitrate Content of Entire Leaf Canopy of Soybeans Across the Growing Season*

Treatments are described in "Materials and Methods."

	Nitrate Reductase Activity								Nitrate		
	1/4X	1X	Soil	LSD[1]	1/4X	1X	Soil	LSD	1/4X	1X	Soil
	μmole NO_2^-/g fresh wt × hr				*μmole NO_2^-/plant × hr*				*μmole NO_3^-/g dry wt*		
June 3	21.30	17.90	12.60	5.90	5.25	4.84	6.85	NS	63	121	184
10	10.36	10.51	6.30	1.68	3.94	4.57	3.45	0.79	48	96	77
17	5.92	7.06	7.03	NS	3.82	5.33	5.84	0.85	97	118	92
24	4.90	5.88	5.22	NS	6.13	9.14	7.41	1.55	39	78	45
July 1	6.28	6.08	5.34	NS	10.45	12.25	6.63	2.57	70	86	54
8	4.35	4.21	4.16	NS	8.28	9.60	7.04	NS	36	56	22
15	4.50	4.32	3.47	NS	9.13	10.21	6.62	2.23	62	78	18
22	6.29	6.64	3.30	1.28	15.33	18.68	5.31	1.88	63	84	16
29	4.74	3.83	2.10	1.83	11.22	10.77	2.56	4.61	81	84	16
Aug. 5	3.80	3.02	1.51	0.58	9.57	8.72	3.00	2.14	57	70	11
12	3.29	2.64	0.92	0.63	8.82	7.33	1.09	1.81	15	18	4
19	2.12	2.11	0.25	0.34	5.03	5.56	0.45	1.59	7	9	2
26	3.40	3.57	0.27	0.43	7.09	6.53	0.40	1.66	3	3	2
Sept. 2	3.10	4.55	0.44	0.46	2.67	3.86	0.49	1.48	7	5	3

[1] LSD (0.05 level) between treatment means when significant F test was obtained with analysis of variance.

Highest levels of activity per g fresh weight per hr existed with the first sampling date when only the unifoliolate leaf was fully emerged. As additional trifoliolates emerged and were included in the average, nitrate reductase activity generally declined throughout the remainder of the growing season. Nitrate reductase activity per g fresh weight per hr of leaf tissue from soil grown plants was significantly lower than from nutrient grown plants on the first two sampling dates and the last seven sampling dates, with differences being nonsignificant from June 17 through July 15 (Table II). Very little difference in nitrate reductase per g fresh weight per hr of plants grown on the two nutrient treatments was evident. Thus it appeared that having a 4-fold difference in nitrate level in the nutrient solutions had little effect on seasonal nitrate reductase activity of this variety of soybeans.

Mean nitrate reductase activity per leaf per hr of the entire leaf canopy was maximal during midseason sampling dates (Table II). The midseason peak in activity per leaf per hr was a reflection of leaf size since maximal activity per g fresh weight per hr was attained at the earliest growth stages. Plants grown on the 1X nutrient treatment had significantly higher nitrate reductase activity (per leaf per hr) than the plants on the 1/4X nutrient treatment on only 3 of the 14 sampling dates. Activity per leaf per hr of soil grown plants was significantly lower than nutrient grown plants on the last eight sampling dates. The magnitude of this difference increased with later sampling dates, and it was evident that nutrient-grown plants had considerably greater potential for nitrate reduction.

Mean nitrate levels of the total leaf canopy were highest at initial sampling stages and declined to low levels by the end of the growing season (Table II). It has been previously shown (5) and was confirmed in the present study (data not shown), that nitrate uptake by the soybean plant from nutrient solution increased rapidly as the plant progressed from the initial flowering stage up through midpod fill. The rate of nitrate uptake declined with growth stages following midpod fill. Because dry matter accumulated faster than nitrate was taken up, nitrate content of the tissue was a function of dilution as well as assimilation over the growing season. Significant positive correlations between nitrate content and nitrate reductase activity per g fresh weight per hr were obtained when comparisons were made at each leaf position across all sampling dates (108 df); r = +0.47, +0.41, and +0.75 for the 1/4X, 1X, and soil treatments, respectively. The correlation was highest with the soil grown plants, where both activity and nitrate concentrations were generally lower than with hydroponically grown plants. Because nitrate reductase was a substrate inducible enzyme and the level of activity was in part a function of nitrate concentration (1), positive correla-

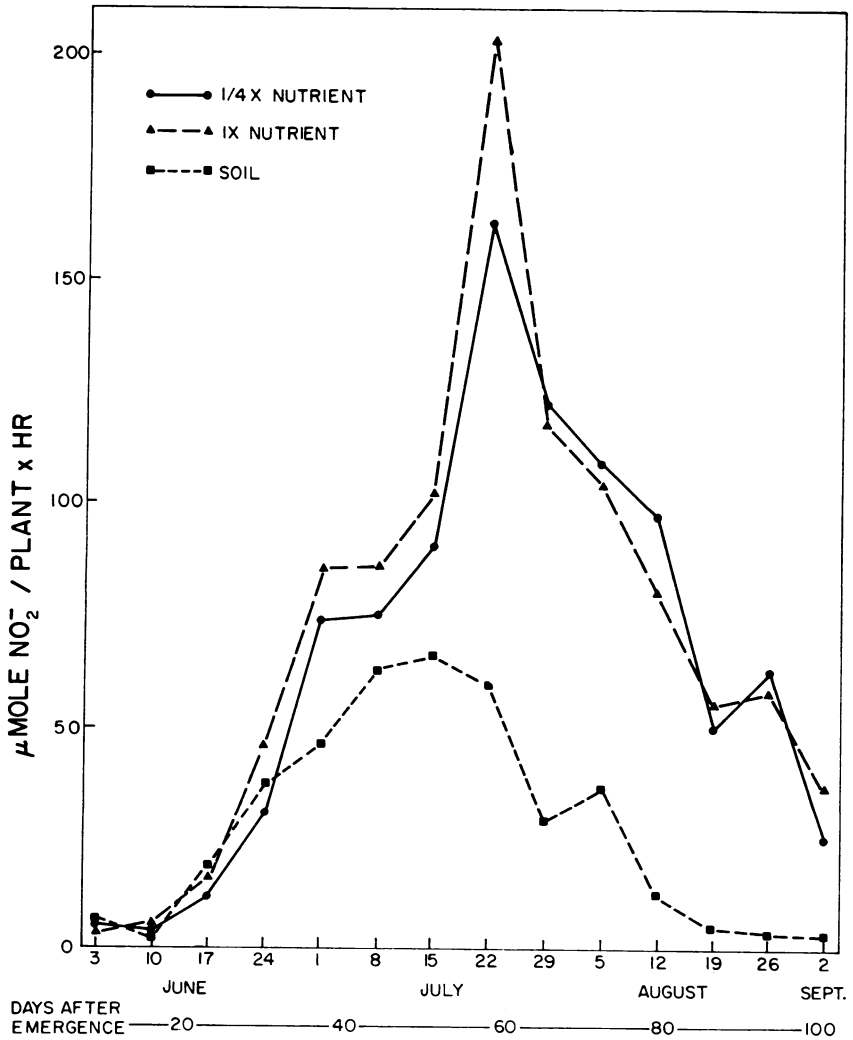

FIG. 6. Seasonal profiles of nitrate reductase activity of soybeans. Values are means of individual measurements at each leaf position within the plant canopy at respective sampling dates. Treatments are described in "Materials and Methods."

tions indicated that in soybeans the decline in nitrate reductase activity through the growing season was partially due to decreased concentrations of nitrate within the plant. The higher levels of both nitrate and nitrate reductase in nutrient grown plants, compared with soil grown plants, indicated that soybeans were responsive to nitrate and nutrient availability to a certain extent. However, the lack of difference in nitrate reductase activity with the two nutrient levels suggested that a genetic limitation in uptake and/or reduction of nitrate may exist.

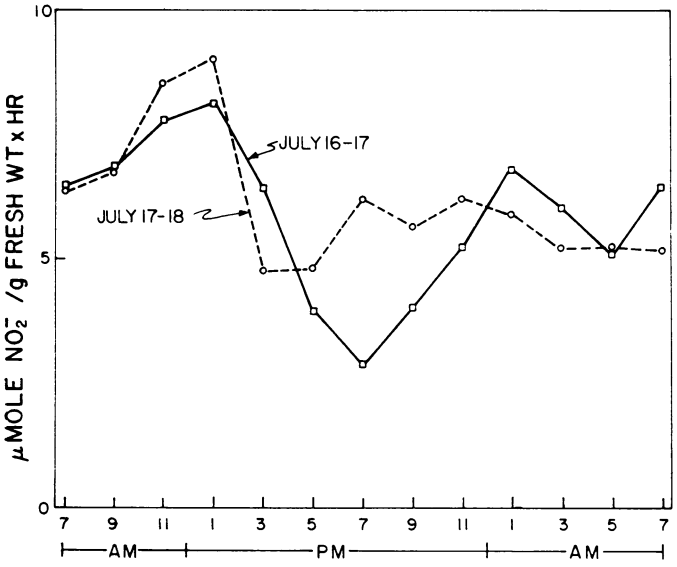

FIG. 7. Diurnal variation in nitrate reductase activity of soybeans. The uppermost fully expanded trifoliolate from field grown plants was assayed. Values are means of eight replications.

When nitrate reductase activity was expressed on a per plant basis (Fig. 6), the maximum activity for nutrient grown plants was much greater than activity of soil grown plants throughout most of the season. Activity per plant per hr closely parallels the nitrogen demand of the plant, with highest activity levels occurring during the critical bean filling stages. In nutrient grown plants the rapid increase in activity from June 17 through July 22 was due to a rapid increase in total leaf fresh weight since activity per g fresh weight per hr declined during the growing season (Table II). Although nitrate reductase activity per plant per hr decreased with maturity, activity was still present at the last sampling date at which time the leaves were rapidly yellowing. Thus, it was evident that the soybean plant had the capacity of supplying considerable reduced nitrogen via nitrate reductase in later stages of pod fill when nutrients and water were available as with the hydroponic treatments. The fact that this variety utilized similar levels of nitrate from hydroponic treatments differing by a 4-fold level of nitrate suggested that soybean varieties with greater potential of nitrate uptake and/or nitrate reductase activity need to be found if a potential response to nitrogen fertilization is realized.

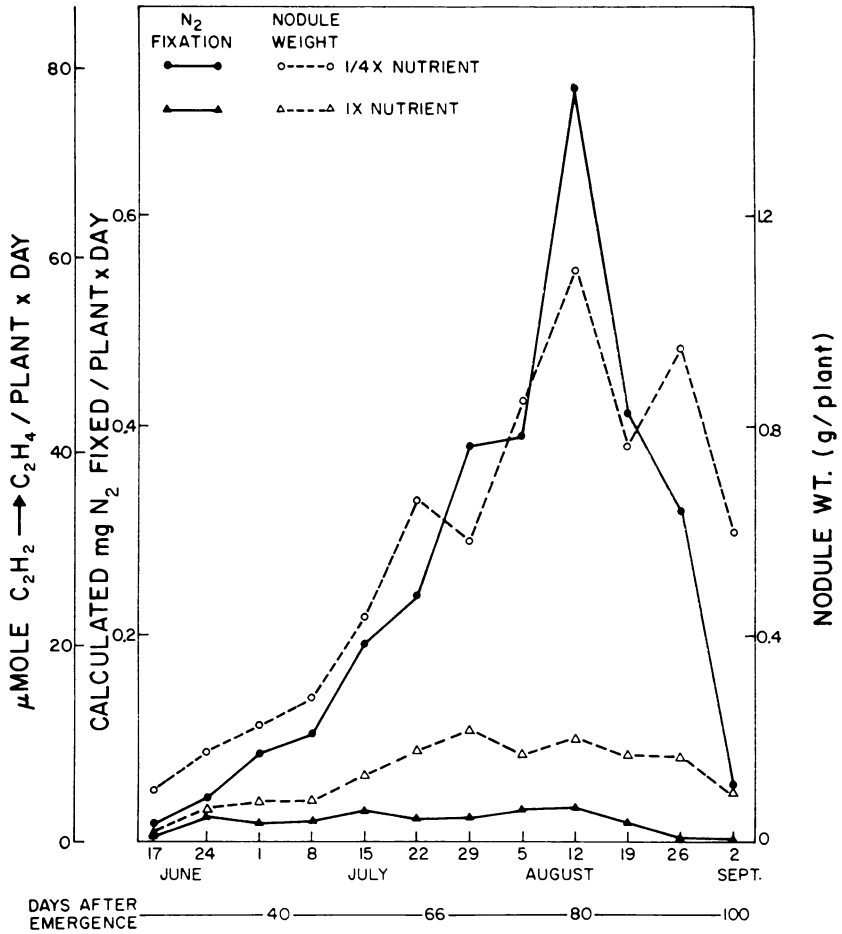

FIG. 8. Seasonal N_2-fixation and nodule weight from soybeans grown in hydroponics. N_2-fixation as estimated from nitrogenase assays measured by the acetylene reduction technique.

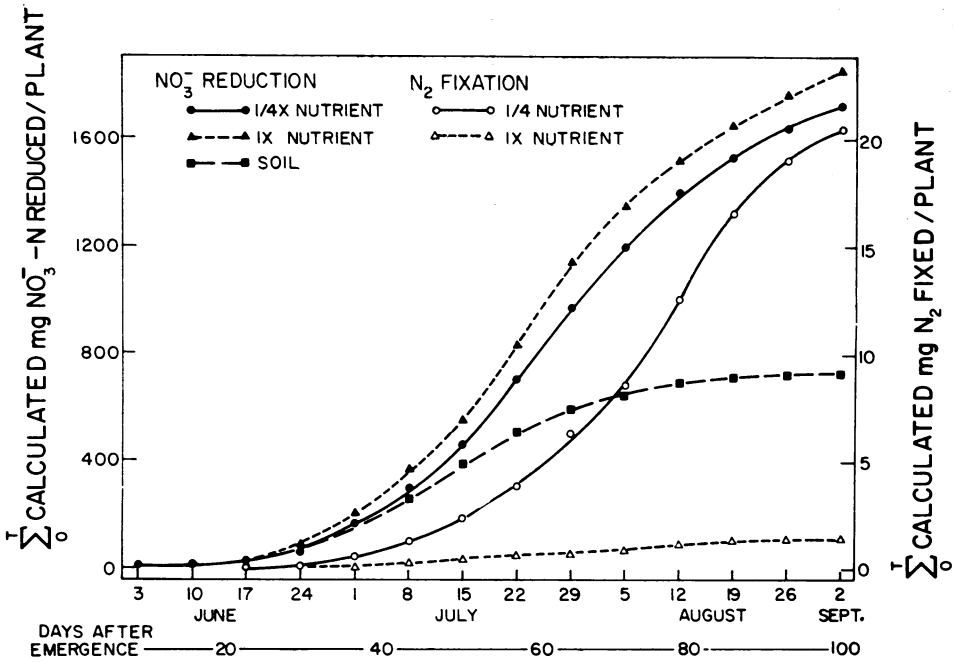

FIG. 9. Cumulative seasonal nitrate reduction via nitrate reductase and N_2-fixation via acetylene reduction technique in soybeans. Treatments are described in "Materials and Methods."

Diurnal Nitrate Reductase Activity. Diurnal patterns of nitrate reductase activity (Fig. 7) were determined to enable calculations of cumulative reduced nitrogen over the growing season via nitrate reductase activity. Nitrate reductase activity was determined for two consecutive 24-hr periods at 2-hr intervals. Maximum activity was attained at 1 P.M. during both 24-hr periods. Activity generally declined during the afternoon hours and reached a minimum during late afternoon or early evening. Nitrate reductase activity appeared to recover slightly during the night from the minimum level. From these and two other sets of data, the activity levels obtained at 10 A.M. with routine sampling were converted to average daily rates for integration of activity over the season.

Nitrogen Fixation. Results of N_2-fixation assays of the hydroponically grown soybeans over the season as measured by the acetylene-reduction technique are indicated in Figure 8. Nitrogen fixation was low on the initial sampling date (June 17) and increased rapidly with subsequent dates in plants grown on the 1/4X nutrient treatment. Peak fixation was reached on August 12 and declined sharply through the last three sampling dates. Fixation values closely paralleled nodule weight with peak nodule weight also being attained on August 12, at which time pods were filling rapidly. The N_2-fixation potential declined more rapidly during the last two sampling dates than did nodule weight and was at a low level by September 2, just prior to maturity. Maximum potential estimated N_2-fixation occurred 3 weeks later than did the peak nitrate reductase activity (Fig. 8). This suggested that the nodules may supply a greater proportion of reduced nitrogen to the plant in later stages of development than is obtained through nitrate reduction. This may be especially true for soil grown soybeans where soil nitrogen levels are low. This view was supported by the low level of nitrate found in the leaves of soil grown plants (Table II). However, in the present study, comparing N_2-fixation with nitrate reductase in hydroponic systems, the overall contribution of nitrate reductase to the nitrogen input of the plant far exceeded fixation input. A low level of N_2-fixation was evident with plants from the 1X nutrient solution which corresponded with the low nodule weight. It was evident that the higher nutrient level (possibly nitrate) was markedly inhibitory to nodulation and N_2-fixation.

Table III. *Seed Yield and Protein Content of Soybeans*

Treatment	Seed Yield	Protein	Seed N
	g/plant	%	*g/plant*
¼X Nutrient	17.3	39.4	1.09
1X Nutrient	19.6	39.4	1.24
Soil	17.4	41.2	1.15

Cumulative Nitrate Reduction and N_2-Fixation. Calculation of cumulative enzymatically reduced nitrate via nitrate reductase over the growing season revealed characteristic sigmoidal curves (Fig. 9). The estimated rate of accumulation of reduced nitrogen was low during the first 35 days after emergence and increased sharply from initial bloom up through midpod fill and declined with the later growth stages. Cumulative estimated nitrate reduction (nitrate reductase assay) was similar with soil and hydroponic grown plants through the first 50 days of growth, whereas thereafter the estimated rate of nitrate reduction was much lower for soil grown plants.

The seasonal potential for nitrate reduction by hydroponic grown plants was more than double that of soil grown plants. This higher potential expressed by hydroponic grown plants was attributed to greater supply of nitrate and unlimited availability of water. The estimated cumulative nitrate reduction over the season with hydroponic grown plants was higher than the final nitrogen content of the seed (1.72 and 1.85 g N per plant estimated [Fig. 9] *versus* 1.09 and 1.24 g N per plant determined in the seed [Table III] for the 1/4X and 1X nutrient treatments, respectively). Unfortunately, nitrogen analysis of the total plant was not obtained in the present study. However, based on estimates by Ohlrogge (9) that 68% of the final N distribution of the entire plant was in the seed, the estimated total N content of the plant and seed in the present

study would agree closely with the estimated potential for nitrate reduction as determined by the nitrate reductase assay.

Sigmoidal curves of N_2-fixation *versus* age were obtained with the 1/4X nutrient treatment (Fig. 9). Greater than 90% of the N_2 fixed occurred post-flowering, which was similar to previous reports (3, 4). The seasonal amount of N_2 fixed by the plants from the 1/4X treatment was less than 10% of the level reported by Hardy *et al.* (4), which indicated that even the 1/4X nutrient treatment was considerably inhibitory to N_2-fixation. Since no estimate of the N_2-fixation by the soil grown plants was made, no direct comparison of the amount of nitrogen supplied to the plants by N_2-fixation and nitrate reductase can be made. However, the nitrogen content of the seed was not markedly different between soil and hydroponically grown plants (Table III). This suggested that N_2-fixation must be making up the difference in nitrogen input in soil grown plants, since estimated nitrate reduction by plants in hydroponics was over twice that in soil grown plants. Thus, the present study appeared to confirm previous observations that the two systems of nitrogen utilization by the soybean plant are either compensating or possibly competing systems.

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