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Relative Abundance of Ureides and Nitrate in Plant Tissues of Soybean as a Quantitative Assay of Nitrogen Fixation[1]

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ABSTRACT

The relationship between the relative abundance of ureides ([ureide-N/ureide-N plus nitrate-N] × 100) in the shoot axis (stems plus petioles), nodulated roots and leaflets of "Bragg" soybean (*Glycine max* [L.] Merrill) and the symbiotic dependence of these plants was examined under glasshouse conditions. Plants, inoculated with effective *Rhizobium japonicum* CB1809, were grown with their roots exposed continuously to a nutrient solution containing either 0, 1.5, 3.0, 6.0 or 12.0 millimolar NO_3-N per liter. Nodulation and N_2-acetylene fixation were correlated inversely with the level of nitrate. Seasonal acetylene reduction profiles for each of the nitrate treatments were integrated and the symbiotic dependence ([N_2 fixed per total plant N] × 100) determined using a conversion ratio of 1.5:1 (acetylene reduced:N_2 fixed), calculated from the zero NO_3 treatment. Examination of the nitrogenous solutes of the shoot axis and nodulated roots showed linear relationships between the relative abundance of ureides and the symbiotic dependence of the plants. Two standard curves, depicting these relationships during vegetative and reproductive growth, were drawn for each plant part. The overriding effect of plant age invalidated any attempt to develop a standard relationship for leaflets. Data from two diurnal studies suggested that relative ureides were insensitive to diurnal fluctuations, thus simplifying sampling procedures. Plant material could be stored at ambient temperatures (20-30°C) for up to 24 h without affecting the relative concentration of ureides and nitrate. It is suggested that the shoot axis provides the most suitable target organ when using this technique as a quantitative assay for N_2 fixation because of ease of sampling of these tissues, especially with field-grown plants.

Examination of the spectra of nitrogenous solutes of plants of soybean (*Glycine max* [L.] Merrill) and cowpea (*Vigna unguiculata* [L.] Walp.) (8, 13, 14, 16) and detailed tracer experiments using ^{15}N and ^{14}C (8, 15) revealed that the ureides, allantoin and allantoic acid, were (a) products of N_2 fixation, (b) responsible for transporting approximately 80% of fixed N from the root nodules of fully symbiotic plants, and (c) present in only minor amounts in plants dependent upon mineral N for nitrogen. These observations suggested that the abundance of ureides in the plant may reflect the symbiotic dependence of the plant and provide a quantitative assay of N_2 fixation (2, 10). Later reports (11, 18), based on analyses of xylem sap (as root bleeding sap) of glasshouse-grown plants for ureides and total N, confirm this, although difficulties in collecting xylem sap in field-grown plants may limit the widespread adoption of this technique. Streeter (20) reported difficulties in collecting root bleeding sap subsequent to the 50 d harvest and resorted to prewatering harvested plants. This may be possible in accessible research plots but is impractical in isolated crops. We have attempted, without success, to collect root bleeding sap from unwatered field-grown plants of soybean, pigeonpea (*Cajanas cajan* [L.] Millsp.), black gram (*Vigna mungo* [L.] Hopper) and green gram (*Vigna radiata* [L.] Wilczek).

Detailed analyses of nitrogenous solutes of plant parts and translocatory streams of cowpea and white lupin (*Lupinus albus* [L.]) showed that the composition of compounds in the shoot axis and, to a lesser extent in leaflets and nodulated roots, reflected that found in the xylem (7). However, the proportions of total soluble-N identified as either ureides or amino compounds were low for both species, averaging between 30 and 36% for roots, 33 and 39% for leaflets, and 56 and 70% for the shoot axis. Unidentified compounds such as nucleotides, low mol wt peptides, alkaloids, and Chl would have comprised the remainder (5). These data suggest that analyses of plant tissues for ureides and nitrate rather than analyses of xylem sap for ureides and total N may facilitate adaption of the ureide technique to field studies.

This paper describes experiments on the relationship between the symbiotic dependence of soybean plants and the relative abundance of ureides and nitrate in plant tissues. Diurnal fluctuations in relative contents of solutes will affect interpretation of standard curves and therefore sampling procedures. As a result, two diurnal studies are reported; one using glasshouse-grown plants, the other using plants grown in the field. Time-lags between sampling of field-grown material and treatment, *e.g.* dehydration, freezing, are common and such delays may affect significantly the relative contents of plant compounds. Accordingly, effects of storage of sampled plant parts prior to treatment are also reported.

MATERIALS AND METHODS

Experiment 1: Effects of Nitrate on Nodulation, N_2-Acetylene Fixation, and Relative Abundance of Nitrogenous Solutes in Plant Parts and Xylem Sap of Soybean. Soybean plants (*G. max* [L.] Merrill, cv. Bragg), inoculated at sowing with effective *Rhizobium japonicum* CB1809, were grown in sand culture in 14-L free-draining pots in a naturally-lit, temperature-controlled glasshouse from November to April. Day temperatures were 30 to 35°C, night temperatures 18 to 25°C. Ten seeds were sown per pot; seedlings were thinned later to three/pot. Positioning of pots and density of plants per pot resulted in an overall density of 20 plants/m^2 of bench.

For the first 3 weeks of plant growth, pots were watered every 2 d with 1 L of a complete nutrient solution containing either 0, 1.5, 3.0, 6.0 or 12.0 mM NO_3/L (as KNO_3 + $Ca[NO_3]_2$). The basal N-free nutrient solution contained 0.25 mM $CaCl_2$, 0.25 mM KCl, 0.5 mM $MgSO_4 \cdot 7H_2O$), 0.13 mM KH_2PO_4, 0.13 mM K_2HPO_4, 23.5 μM Fe-EDTA, and micro-nutrients (4). Subsequently, each pot received 2 L of the appropriate nutrient solution daily. The frequency of application of the nutrient solutions resulted in a regular and thorough flushing of the pots and ensured uniform levels of N and other elements in the rooting medium. Conditions

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of plant culture and concentrations of nitrate used in the nutrient solution were selected to provide plants comparable in size to those grown in the field and ranging from complete dependence upon symbiotic N (zero NO_3) to almost total reliance upon mineral N (12.0 mM NO_3).

Duplicate experiments were combined in this study relating ureide and nitrate contents of plant parts to symbiotic dependence. Statistical analyses of all data showed no significant differences between experiments in any of the characters examined. Samples of eight to twelve plants for each of the five nitrate treatments were harvested at noon at 35, 49, 61, 77, 90, and 103 days after sowing (set 1) and 35, 49, 62, 77, 91 and 110 days in Set 2. Physiological development of plants was noted according to the scheme of Fehr *et al.* (3) (Table I). Plants were separated immediately into nodulated roots and shoots. N fixation activity was assessed for each treatment using the acetylene reduction assay (21). Decapitated roots (with nodules intact) were placed in 1.25 L gas-tight preserving jars (4 jars/treatment). A 125 cm^3 volume of air was removed from each vessel and replaced with an equal volume of acetylene. Nodulated roots were incubated for 30 min. Duplicate 1-cm^3 samples of gas were then withdrawn from each vessel and analyzed for ethylene by gas chromatography using a flame ionization detector. Roots and nodules were separated, dried at 80°C for 48 h, and weighed. They were recombined and ground to pass through a 1-mm sieve. Shoots were dried, weighed, and divided into two samples. One half was separated into the shoot axes (stems plus petioles) and leaflets for grinding as above. The other half was left intact and ground. Total N values of shoots and nodulated roots were determained by Kjeldahl analysis. Samples (1 g) of shoot axes, leaflets, and nodulated roots were extracted with 25 ml boiling water for 2 min (1). The extracts were filtered, made up to volume (50 ml), and stored at −15°C to be analyzed later for nitrate by autoanalysis (6) and ureides by TLC (9) and colorimetrically as the phenylhydrazone of glyoxalate (23).

Xylem exudate was collected as bleeding sap from the cut base of plant stems on day 51 (growth state R1) (17). Sap was collected for a 20-min period at noon and frozen immediately to be analyzed for ureides and nitrate as detailed above.

Experiment 2: Diurnal Effects on Relative Abundance of Nitrogenous Solutes in Plant Parts. Plants were grown under glasshouse and field conditions and examined for diurnal fluctuations in solute contents and N_2-acetylene fixing activity. Cultural conditions of glasshouse plants were identical to those described in experiment 1 except that all plants received the basal nutrient solution incorporating 1.5 mM NO_3/L. Groups of twelve plants were sampled every 3 h, commencing at the beginning of the photoperiod 42 d after sowing (growth stage V7) and terminating at the end of the following night period. Plants were separated into nodulated roots, stems plus petioles and leaflets. Acetylene reduction assays were done on the nodulated roots; four root systems in each of three incubation vessels (as described previously). All plant parts were then dried at 80°C for 48 h, ground to pass through a 1-mm sieve, and analyzed for ureides and nitrate as described previously.

Plants used in the field study were harvested from uniform seed increase plots of variety 'Dickie,' inoculated at sowing with *R. japonicum* CB1809 and irrigated throughout growth. Soil type was a fertile, grey clay of heavy texture (pH 8.0). Sampling and assay procedures were similar to those described above except that groups of 20 plants were harvested at 3 to 4 h intervals at the R5 stage of growth. For the acetylene reduction assays, 10 incubation vessels, containing two nodulated roots/vessel, were used at each sampling time.

Table I. *Age and Physiological Development of Soybean Plants at each Harvest in Experiment 1*

Two sets of plants were grown under glasshouse conditions and destructively harvested at the times shown. Data from the two sets of plants were combined. Physiological development of the plants at harvest was noted according to the scheme of Fehr *et al.* (1971). Flowering occurred 50 d after sowing.

Days after Sowing	Set of Plants	Physiological Development
35	1 and 2	V4
49	1 and 2	V8
61, 62	1 and 2	R2
77	1 and 2	R5
90	1	R5
91	2	R6
103	1	R6
110	2	R7

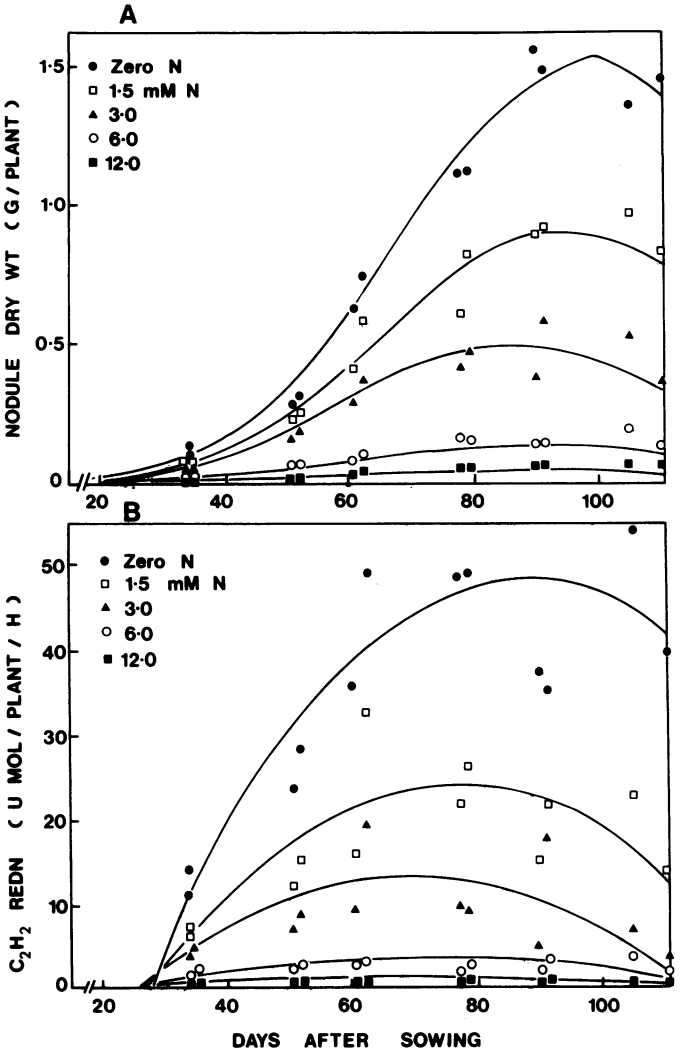

FIG. 1. Changes during growth of soybean in (A) dry weight of nodule tissue/plant for each of the five nitrate treatments (each point is the mean of four replicates); (B) acetylene (C_2H_2) reduction activity. Curves of best fit are drawn, described by the following equations:

$$\text{zero } NO_3\ y = -53.734 + 2.259x - 0.0124884x^2$$

$$1.5 \text{ mM } NO_3 = -31.896 + 1.446x - 0.0093899x^2$$

$$3.0 \text{ mM } NO_3 = -17.533 + 0.854x - 0.0060644x^2$$

$$6.0 \text{ mM } NO_3 = -1.857 + 0.097x - 0.0005526x^2$$

$$12.0 \text{ mM } NO_3 = -0.366 + 0.023x - 0.0001382x^2$$

Each point is the mean of four replicates.

Table II. *Estimation of Symbiotic Dependence of Glasshouse-Grown Soybean Plants Exposed to Five Levels of Nitrate*

NO_3 Treatment	Seasonal Acetylene Reduction	N Uptake	N_2 Fixed[b]	Plant N as N_2 Fixed
mM/l	*mmol C_2H_2/plant*[a]	*mmol N_2/plant*	*mmol/plant*	*%*
0	69.13	42.25	45.25	100
1.5	35.15	47.00	22.97	49
3.0	17.85	49.60	11.67	23
6.0	3.84	49.68	2.51	5
12.0	1.08	61.04	0.71	1

[a] A diurnal correction factor of 1.0 was used. All acetylene reduction assays were done at noon, when activity was equal to the mean activity for assays throughout the diurnal period (see Fig. 5C).

[b] Calculated using the conversion ratio of 1.5:1 (69.13:45.25) to convert acetylene reduced to N_2 fixed. Assumes that the conversion ratio calculated for the zero NO_3 treatment can be used for the other four treatments.

Experiment 3: Effects of Storage on Relative Abundance of Nitrogenous Solutes in Plant Parts. A group of 40 uniform plants was harvested from a field plot of 'Bragg' soybeans at growth stage R5. One, 2, 4, and 24 h after harvest, samples of 10 plants were partitioned into stems plus petioles, leaflets and nodulated roots, dried at 80°C for 48 h and analyzed for ureides and nitrates after extraction. Temperature during the period of storage fluctuated from 20 to 30°C. Time lapses between plant harvest and drying and storage temperatures were considered to be typical of conditions to which plants harvested in field studies might be subjected.

RESULTS AND DISCUSSION

Nodulation of and N_2 fixation by the soybean plants were effectively regulated by the nitrate treatments. Exposure of plants to the highest level of nitrate (12.0 mM) resulted in the lowest production of nodular tissue (Fig. 1A) and lowest rates of N_2-acetylene fixing activity (Fig. 1B). The completely symbiotic plants (zero NO_3) produced the most nodule tissue and showed the highest acetylene-reducing activity. Maximum dry weight of nodules was recorded for each treatment during the R5 to R6 stage of development. When compared with the fully symbiotic plants, nodule mass/plant was reduced by 25 to 40% with addition of 1.5 mM NO_3, 40 to 67% (3.0 mM NO_3), 77 to 91% (6.0 mM NO_3), and 83 to 97% (12.0 mM NO_3).

Nitrogen fixation by, and hence symbiotic dependence of, each of the five nitrate treatments was assessed using the acetylene reduction assay. There are recognized difficulties in using this technique quantitatively in field studies (19) but under controlled conditions, as with pot-grown plants in glasshouse experiments, no particular problems have been recognized. Acetylene reduction activity was recorded at the first harvest (35 d), reached maxima in all treatments between 70 and 90 d (R4 to R6), then declined. Activity was inversely correlated with the level of nitrate; the highest activity recorded was from the zero NO_3 plants. Equations describing seasonal profiles of acetylene reduction for the five nitrate treatments (legend, Fig. 1B) were integrated and the amounts of acetylene reduced/plant over the whole growth cycle determined after correction for diurnal variation in acetylene reduction activity (Table II). For each treatment, total N uptake/plant was also calculated and compared with N_2 fixed/plant as estimated from the integrated seasonal profiles and converting acetylene reduced to N_2 fixed using the ratio of 1.5:1 (see footnote, Table II). Symbiotic dependence of plants (N_2 fixed as percentage of total plant N) for the five nitrate treatments was then determined. The acetylene:N_2 ratio was low when compared to the theoretical ratio of 3 to 4.5:1 (21). Decapitation of soybean plants prior to assay resulted in decreases of between 55 and 63% in acetylene reduction activity (12, 22). In the present study, nodulated roots rather than whole plants were used for assay because

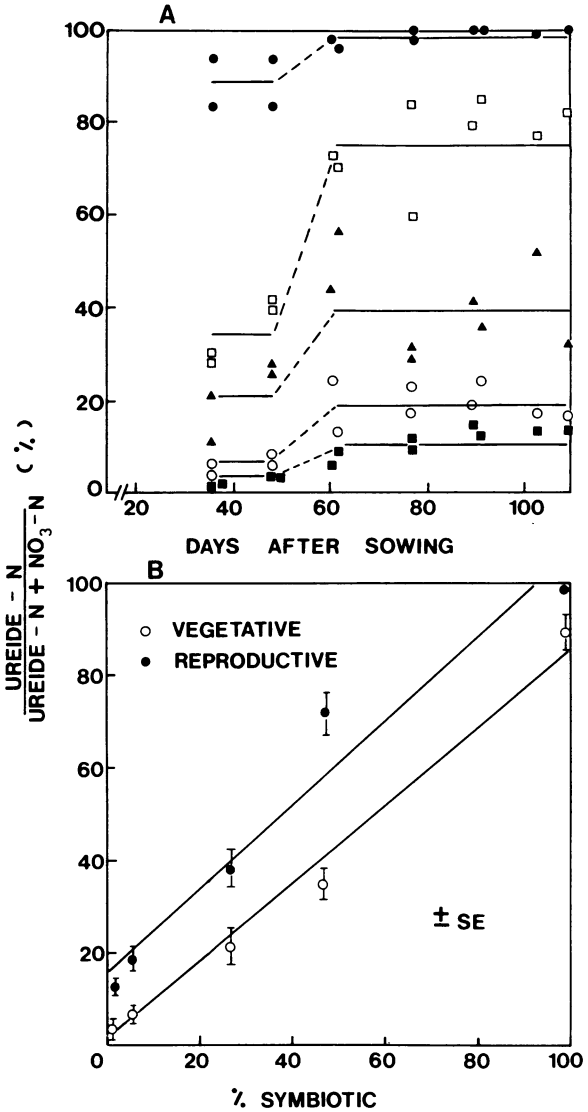

FIG. 2. A, effect of nitrate on the relative abundance of ureides (expressed as ureide-N as a percentage of ureide-N plus nitrate-N) in shoot axes of soybean during growth. Nitrate treatments are: (●), zero NO_3; (□), 1.5 mM NO_3; (▲), 3.0 mM NO_3; (○), 6.0 mM NO_3; and (■), 12.0 mM NO_3. Note the increase in relative abundance of ureides for each treatment subsequent to the first two (vegetative) harvests. B, relationship between the relative abundance of ureides in shoot axes and symbiotic dependence of soybean plants. The two straight lines cover vegetative growth (○) and reproductive growth (●). Vertical lines indicate SEM.

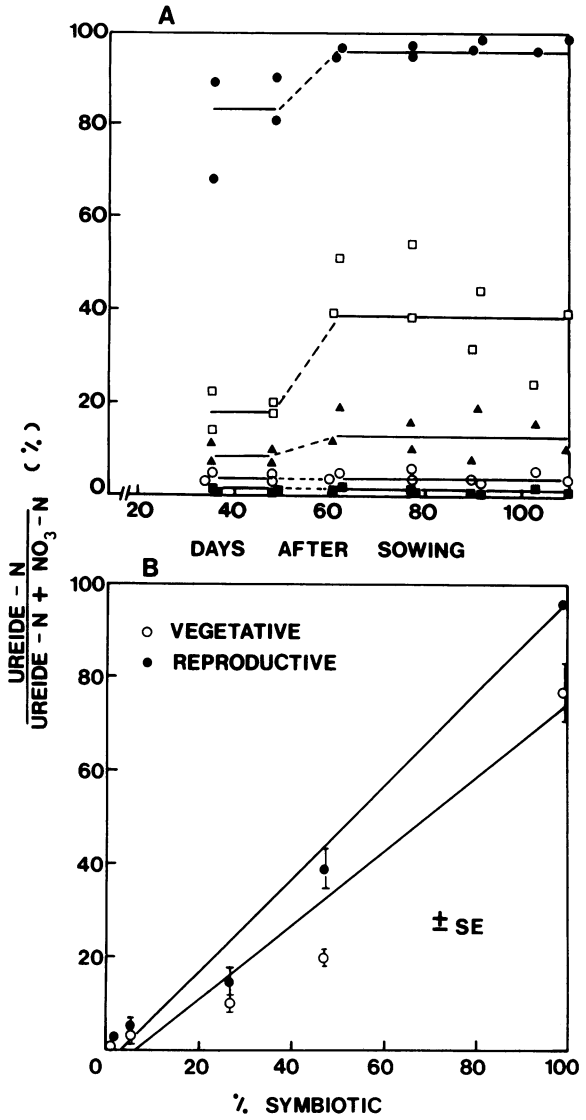

FIG. 3. A, effect of nitrate on the relative abundance of ureides in nodulated roots of soybean during growth. Nitrate treatments are: (●), zero NO_3; (□), 1.5 mM NO_3; (▲), 3.0 mM NO_3; (○), 6.0 mM NO_3; and (■), 12.0 mM NO_3. B, relationship between relative abundance of ureides in nodulated roots and symbiotic dependence of soybean plants. The two straight lines cover vegetative growth (○) and reproductive growth (●). Vertical lines indicate SEM.

of the problems of accommodating large volumes of shoot in the assay vessel. Higher rates of activity from whole plants would have resulted in a significantly higher conversion ratio. However, consistent assay procedures for all treatments validate the use of the calculated ratio of 1.5:1 (Table II).

Examination of the solutes of the stems plus petioles showed that the relative abundance of ureides (expressed as ureide-N as a percentage of ureide-N plus nitrate-N) decreased with increasing levels of nitrate (Fig. 2A). For each treatment, there was an effect of plant age due to increased concentrations of ureides and lower nitrate levels in tissues harvested during reproductive growth when compared to tissues harvested during vegetative growth. For example, plants fed 3.0 mM NO_3 showed mean concentrations of ureides of 9.4 and 20.6 μmol/g dry weight during vegetative and reproductive periods, respectively. Mean nitrate contents for the same two periods of growth were 149 and 138 μmol/g dry weight. As a result, the relationships for the two periods between the relative abundance of ureides in the shoot axis and the symbiotic dependence of the plants are presented (Fig. 2B) and are described by the following equations:

$$\text{vegetative growth, } y = 1.161 + 0.838x \quad R^2 = 0.95$$
$$\text{reproductive growth, } y = 16.800 + 0.893x \quad R^2 = 0.90$$

Effects of nitrate on the relative abundance of ureides in nodulated roots are shown in Figure 3A. Again, relative abundance of ureides were not constant throughout growth and two phases are recognized. Standard curves were drawn (Fig. 3B), described by the following equations:

$$\text{vegetative growth, } y = -5.411 + 0.797x \quad R^2 = 0.89$$
$$\text{reproductive growth} y = -3.518 + 0.963x \quad R^2 = 0.97$$

Examination of the effects of nitrate on the relative abundance of ureides in leaflet tissues suggests an overriding effect of plant age (Fig. 4). The zero nitrate treatment was the most consistent with a relative abundance of ureides of almost 100% for the whole growth cycle. With the 3.0, 6.0, and 12.0 mM NO_3 treatments, relative ureides ranged from a low as 7% at the first harvest (35 d) to almost 100% by day 110. As with the other plant parts, the effect of plant age was caused by increasing concentrations of ureides and declining concentrations of nitrate. With the 3.0 mM NO_3 treatment, concentrations of ureides in leaflets ranged from 3.7 μmol/g dry weight (mean of 35 d harvests, sets 1 and 2) to 49.2 μmol/g dry weight at the 90 and 91 d harvests. Concentrations of nitrate for the same tissues were 60.3 (35 d) and 52.0 (90/91 d) μmol/g dry weight. With the 12.0 mM NO_3 treatment, where plants were relying on N_2 fixation for 1% of their requirements for N, elevated levels of ureides in leaflets (25.5 μmol/g dry weight at the 90 d harvest) could not have been associated with N_2 fixation and exemplifies a potentially misleading situation if leaflets were used as the target organs to quantify the symbiotic dependence of the plants.

The ureide and nitrate contents of xylem sap collected from plants in early flowering (R1) are shown in Table III. The combined N contents of the two solutes were remarkably consistent for the five levels of nitrate, ranging from 130 to 188 μg/ml. Assuming that 80% of xylary N is contained in ureides and nitrate (10, 18), total concentrations of xylem sap N in the present study

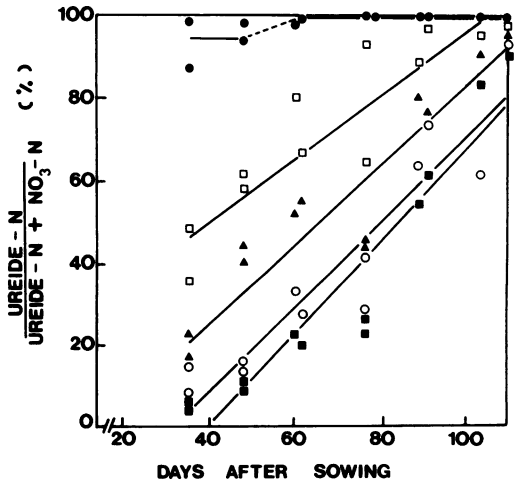

FIG. 4. Effect of nitrate on the relative abundance of ureides in leaflets of soybean during growth. Nitrate treatments are: (●), zero NO_3; (□), 1.5 mM NO_3; (▲), 3.0 mM NO_3; (○), 6.0 mM NO_3; and (■), 12.0 mM NO_3. Note the effect of plant age as well as nitrate treatment on relative abundance of ureides.

were almost identical to the concentrations of approximately 200 μg/ml reported by Israel and McClure (10) for glasshouse grown plants of soybean.

An important consideration in determining sampling procedures is the effect of diurnal fluctuations on the relative abundance of ureides which, if present, would necessitate the use of correction factors to determine symbiotic dependence of test plants. Diurnal patterns of relative ureides in plant tissues and acetylene reduction of both glasshouse and field-grown soybean were studied (Fig. 5). The relative abundance of ureides displayed no diurnal variation in either glasshouse or field experiments and, as occurred with the previous experiments (Figs. 2, 3, and 4), was highest in leaflet tissues and lowest in nodulated roots. Previous work (11) had suggested little change in relative ureide contents of xylem sap during a 24-h period. The relative abundance of ureides (Fig. 5A) and low rates of acetylene reduction activity (Fig. 5C) in the glasshouse-grown plants reflected the partial symbiotic dependence of these plants, watered continuously with a nutrient solution

Table III. *Effect of Nitrate on the Concentrations of Ureides and Nitrate in Xylem Exudate from 51-Day Soybean Plants*

Plants were grown under glasshouse conditions and were in early flowering (R1) at harvest.

NO_3 Treatment	Plant N as N_2 fixed	Concn. of Xylary Ureides	Concn. of Xylary Nitrate	Relative[a] Abundance Ureides
mM/l	%	*μmol/ml*		
0	100	3.1	0.8	94
1.5	49	1.8	3.3	69
3.0	23	1.1	4.9	47
6.0	5	0.5	8.2	20
12.0	1	0.3	12.2	9

[a] Expressed as ureide-N as a percentage of ureide-N plus nitrate-N.

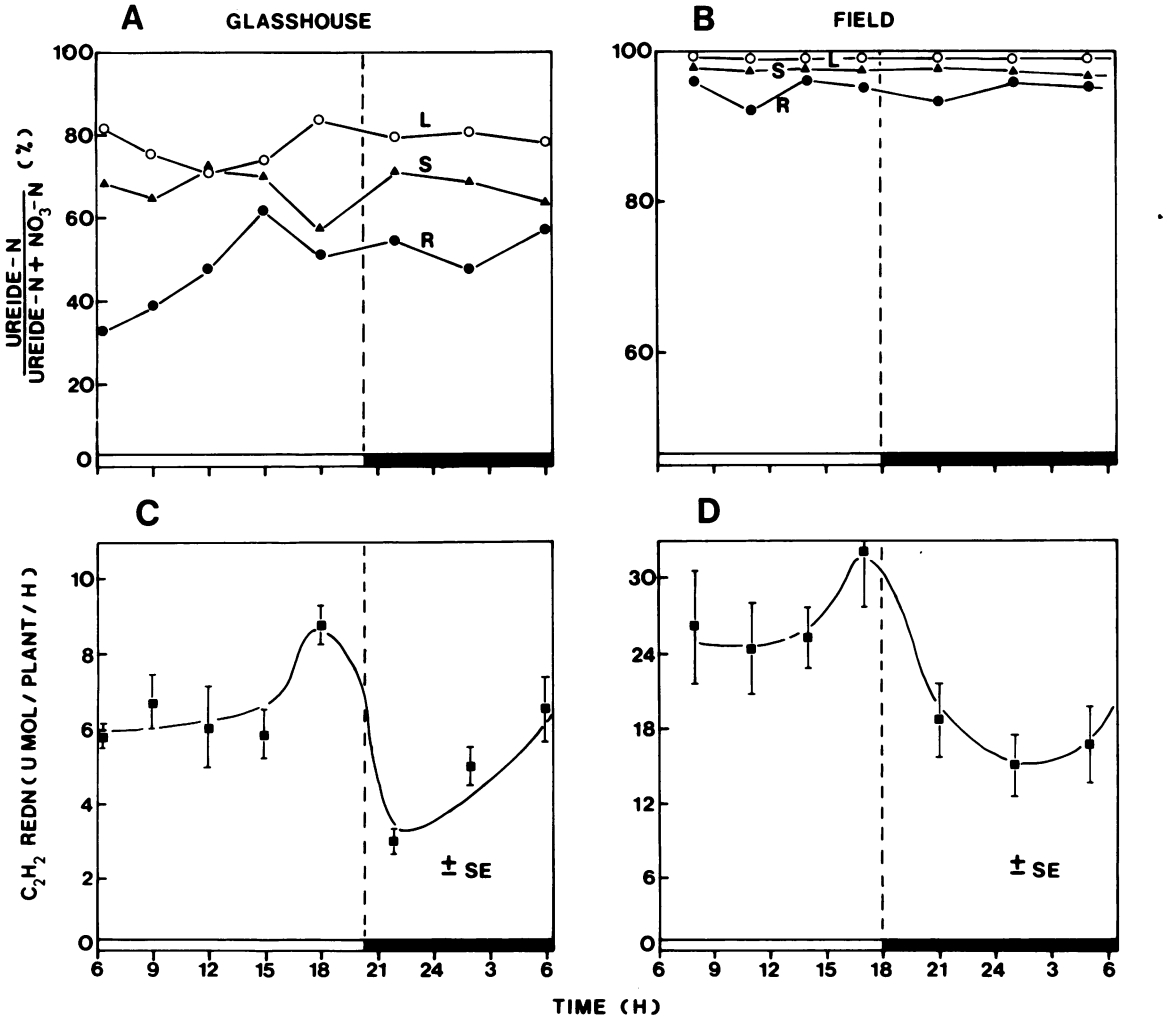

FIG. 5. Diurnal patterns of relative abundance of ureides in leaflets (L), shoot axes (S), and nodulated roots (R) of: A, glasshouse-grown soybean plants; B, field-grown soybean plants. Diurnal patterns of acetylene (C_2H_2) reduction activity by: C, glasshouse-grown soybean plants; D, field-grown soybean plants. Vertical lines indicate SEM.

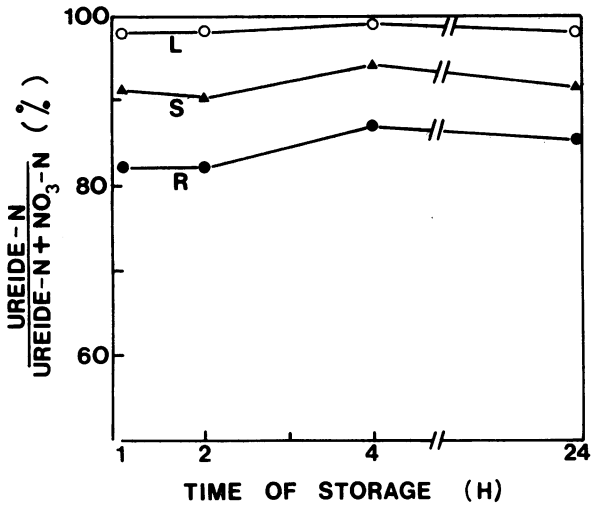

FIG. 6. Effect of storage of soybean plants prior to drying on relative abundance of ureides in leaflets (L), shoot axes (S), and nodulated roots (R).

containing 1.5 mM NO_3/L. Patterns of ureides in tissues of field-grown plants (Fig. 5B) and high rates of acetylene reduction (Fig. 5D) suggested an almost total dependence upon N_2 fixation by these plants. Diurnal acetylene-reducing activity peaked at 1800 h for both glass house and field grown plants.

The time-lag between sampling of plants, especially from field plots, and oven drying of plant tissues prior to extraction and analyses may be considerable and differential degradation of solutes may occur leading to false conclusions. Data on effects of storage prior to drying suggest that plant material can be stored for up to 24 h with no obvious effects on relative ureides in any of the tissue extracts (Fig. 6).

To conclude, analyses of tissue extracts of soybean showed that the relative abundance of ureides and nitrate in the shoot axes and nodulated roots reflected the symbiotic dependence of the plant. For each plant part, two phases in the growth cycle of the plants were recognized and, as a consequence, two standard curves drawn. With leaflets, the significant effect of plant age invalidated any attempt to relate the relative abundance of ureides and nitrate to N_2 fixation. Relative ureides were consistent over a diurnal period, thus simplifying harvest procedures and eliminating the need for diurnal correction. Plants could be stored for up to 24 h prior to drying. The shoot axis (stem and petioles) provided the most useful target organ for tissue analyses when this technique was used as a quantitative assay. Sampling of stems and petioles in field plants is simple and total recovery of tissues is not a problem as with below ground organs.

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LITERATURE CITED

1. AVERY RJ, M ROBERTS, HA SMITH 1973 Preparation of plant, soil and fertilizer digests and extracts. *In* O Talibudeen, ed, Analysis of Crops, Soils, and Fertilizers. Rothamsted Esp Stn, Harpenden, UK, p 16
2. DAY JM, RJ ROUGHLEY 1978 Ureides (allantoin and allantoic acid) in xylem exudate as a measure of nitrogen fixation in legumes. Rothamsted Exp Stn Rep Pt 1, pp 241–242
3. FEHR WR, CE CAVINESS, DT BURMOOD, JS PENNINGTON 1971 Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci 11: 929–931
4. GIBSON AH 1980 Methods for legumes in glasshouses and controlled environment cabinets. *In* FJ Bergersen, ed, Methods for Evaluating Biological Nitrogen Fixation. John Wiley and Sons, Brisbane, pp 139–184
5. HEGARTY MP, PJ PETERSON 1973 Free amino acids, bound amino acids, amines and ureides. *In* GW Butler, RW Bailey, eds, Chemistry and Biochemistry of Herbage, Vol 1. Academic Press, London, pp 2–62
6. HENZELL EF, I VALLIS, JE LINDQUIST 1968 Automatic colorimetric methods for the determination of nitrogen in digests and extracts of soils. Int Congr Soil Sci Trans 9th (Adelaide, Aust) 3: 513–520
7. HERRIDGE DF 1977 Carbon and nitrogen nutrition of two annual legumes. PhD thesis. University of Western Australia, Perth
8. HERRIDGE DF, CA ATKINS, JS PATE, RM RAINBIRD 1978 Allantoin and allantoic acid in the nitrogen economy of the cowpea (*Vigna unguiculata* (L.) Walp.) Plant Physiol 62: 495–498
9. HERRIDGE DF, JS PATE 1977 Utilization of net photosynthate for nitrogen fixation and protein production in an annual legume. Plant Physiol 60: 759–764
10. MCCLURE PR, DW ISRAEL 1979 Transport of nitrogen in the xylem of soybean plants. Plant Physiol 64: 411–416
11. MCCLURE PR, DW ISRAEL, RJ VOLK 1980 Evaluation of the relative ureide content of xylem sap as an indicator of N_2 fixation in soybeans. Plant Physiol 66: 720–725
12. MAGUE TH, RH BURRIS 1972 Reduction of acetylene and nitrogen by field-grown soybeans. New Phytol 71: 275–286
13. MATSUMOTO T, Y YAMAMOTO, M YATAZAWA 1976 Role of root nodules in the nitrogen nutrition of soybeans. II. Fluctuation in allantoin concentration of the bleeding sap. J Sci Soil Manure Jpn 47: 463–469
14. MATSUMOTO T, M YATAZAWA, Y YAMAMOTO 1977 Distribution and change in the contents of allantoin and allantoic acid in developing nodulating and non nodulating soybean plants. Plant Cell Physiol 18: 353–359
15. MATSUMOTO T, M YATAZAWA, Y YAMAMOTO 1977 Incorporation of ^{15}N into allantoin in nodulated soybean plants supplied with $^{15}N_2$. Plant Cell Physiol 18: 459–462
16. MATSUMOTO T, M YATAZAWA, Y YAMAMOTO 1977 Effects of exogenous nitrogen-compounds on the concentrations of allantoin and various constituents in several organs of soybean plants. Plant Cell Physiol 18: 613–624
17. PATE JS 1973 Uptake, assimilation and transport of nitrogen compounds by plants. Soil Biol Biochem 5: 109–119
18. PATE JS, CA ATKINS, ST WHITE, RM RAINBIRD, KC WOO 1980 Nitrogen nutrition and xylem transport of nitrogen in ureide-producing grain legumes. Plant Physiol 65: 961–965
19. PHILLIPS DA, JP BENNETT 1978 Measuring symbiotic nitrogen fixation in rangeland plots of *Trifolium subterraneum* L. and *Bromus mollis* L. Agron J 70: 671–674
20. STREETER JG 1972 Nitrogen nutrition of field-grown soybean plants. I. Seasonal variations in soil nitrogen and nitrogen composition of stem exudate. Agron J 64: 311–314
21. TURNER GL, AH GIBSON 1980 Measurement of nitrogen fixation by indirect means. *In* FJ Fergusen, ed, Methods for Evaluating Biological Nitrogen Fixation. John Wiley and Sons, Brisbane, pp 111–138
22. WYCH RD, DW RAINS 1978 Simultaneous measurement of nitrogen fixation estimated by acetylene-ethylene assay and nitrate absorption by soybeans. Plant Physiol 62: 443–448
23. YOUNG EG, CF CONWAY 1942 On the estimation of allantoin by the Rimini-Schryver reaction. J Biol Chem 142: 839–853