Plant Physiol. (1980) 66, 720–725
0032-0889/80/66/0720/06/$00.50/0

Evaluation of the Relative Ureide Content of Xylem Sap as an Indicator of N_2 Fixation in Soybeans

GREENHOUSE STUDIES[1]

Received for publication March 21, 1980 and in revised form June 6, 1980

PETER R. MCCLURE[2], DANIEL W. ISRAEL[3], AND RICHARD J. VOLK[2]
[2]*Department of Soil Science, North Carolina State University, Raleigh, North Carolina 27650*[3]
United States Department of Agriculture, Science and Education Administration, Agricultural Research, Department of Soil Science, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

The use of the relative ureide content of xylem sap [(ureide-N/total N) × 100] as an indicator of N_2 fixation in soybeans (Merr.) was examined under greenhouse conditions. Acetylene treatments to inhibit N_2 fixation were imposed upon the root systems of plants totally dependent upon N_2 fixation as their source of N and of plants dependent upon both N_2 fixation and uptake of exogenous nitrate. Significant decreases in the total N concentration of xylem sap from plants of the former type were observed, but no significant decrease was observed in the total N concentration of sap from the latter type of plants. In both types of plants, acetylene treatment caused significant decreases in the relative ureide content of xylem sap. The results provided further support for a link between the presence of ureides in the xylem and the occurrence of N_2 fixation in soybeans. The relative ureide content of xylem sap from plants totally dependent upon N_2 fixation was shown to be insensitive to changes in the exudation rate and total N concentration of xylem sap brought about by diurnal changes in environmental factors. There was little evidence of soybean cultivars or nodulating strains affecting the relative ureide content of xylem sap. 'Ransom' soybeans nodulated with *Rhizobium japonicum* strain USDA 110 were grown under conditions to obtain plants exhibiting a wide range of dependency upon N_2 fixation. The relative ureide content of xylem sap was shown to indicate reliably the N_2 fixation of these plants during vegetative growth using a ^{15}N method to measure N_2 fixation activity. The use of the relative ureide content of xylem sap for quantification of N_2 fixation in soybeans should be evaluated further.

Soybean plants acquire N from their environment by the uptake of nitrogenous compounds from the soil solution and by the symbiotic fixation of atmospheric N_2 within their root nodules. Although several methods have been developed to estimate the contribution made by N_2 fixation to the total N accumulation of soybean and other leguminous plants, a need still exists for a rapid, inexpensive, and quantitative method that will separate the contributions made by the uptake of soil and fertilizer N and the fixation of N_2 in field-grown soybeans.

The C_2H_2 reduction assay (6, 10, 22) provides a sensitive, relatively inexpensive, and simple method for measuring instantaneous nitrogenase activity. However, application of this method to the quantitative measurement of seasonal profiles of N_2 fixation in field situations has a number of shortcomings. The procedure underestimates N_2 fixation due to the negative effect of root excision on C_2H_2 reduction (7, 18) and the difficulty in extracting all nodules from the soil, especially at later developmental stages when nodules may exist on lateral roots at some distance from the root crown. Overestimations arise when roots are extracted from the soil due to decreased resistance to gaseous diffusion (7) and when legume root nodules which evolve H_2 under ambient conditions are assayed (23). Phillips and Bennett (21) suggested that the accuracy of the method can be improved by measuring both C_2H_2 reduction and H_2 evolution at each sampling. However, frequent, if not continuous, sampling is mandatory because both C_2H_2 reduction (9, 25) and H_2 evolution (2, 3) are affected by environmental factors. The observation of significant variation in the ratio of C_2H_2 reduced to N_2 fixed among experiments with detached soybean nodules led Bergerson (1) to conclude that caution should be observed when applying C_2H_2 reduction measurements to the quantification of N_2 fixation and that the method is best suited for qualitative comparisons of nitrogen-fixing systems.

The most precise methods for quantitatively determining N_2 fixation involve using ^{15}N-labeled N_2 or nitrate and ammonium ions in culture media free of other N. The use of ^{15}N-labeled N_2 is not practical on a field plot scale and, although the addition of labeled nitrate or ammonium to a field soil gives valid estimates of fertilizer-derived N, it does not separate the symbiotically fixed and soil-derived N.

The ^{15}N A-value technique proposed by Fried and Broeshart (8) has received attention as a method which will separate the contributions made by soil, fertilizer, and symbiotic fixation to the N input of field-grown leguminous plants (5, 21). The method involves applying different levels of ^{15}N-labeled fertilizer to nodulating and non-nodulating isolines of leguminous plants. The method assumes that the relative amounts of soil-derived N and fertilizer-derived N are the same in the tissue of both isolines. If the geometry or extensiveness of the root systems of the two isolines were not similar and, as a consequence, one isoline exploited relatively more of the soil N which was devoid of labeled N, this assumption would not be valid. To our knowledge, tests of the assumption, under field or lysimeter conditions, have not been conducted.

Although the ^{15}N techniques provide the most precise quantitative determinations of N_2 fixation, the costs of obtaining a mass

[1] Cooperative investigation of the United States Department of Agriculture, Science and Education Administration, Agricultural Research and the North Carolina Agricultural Research Service, Raleigh, N. C. Paper No. 6418 of the Journal Series of the North Carolina Agricultural Research Service at Raleigh. The results of this paper form part of the Ph.D. dissertation of P. R. M.

spectrometer for isotope ratio analysis or of paying for isotope ratio analysis may be prohibitive to some investigators. Therefore, the availability of a less expensive method with similar precision would be desirable.

The occurrence of the ureides, allantoin and allantoic acid, as dominant nitrogenous compounds in the xylem sap of nodulated soybeans (11, 12, 15, 16, 26) has led to the hypothesis that the relative ureide content of soybean xylem sap may be a useful indicator of the contribution made by N_2 fixation to the N input of the soybean plant. The investigation reported here was undertaken to examine more closely the relationship between N_2 fixation and the relative ureide content of soybean xylem sap and to calibrate the relative content of xylem sap with the relative contribution of N_2 fixation to the total N input of greenhouse-grown soybean plants as measured by a ^{15}N tracer method.

MATERIALS AND METHODS

Experiment 1: C_2H_2 Inhibition of N_2 Fixation. Soybean plants (*Glycine max* [L.] Merr., 'Davis') were grown in an unshaded greenhouse for 52 days from late January to mid-March, 1979. Metal halide lamps (400 μE m^{-2} s^{-1} PAR at bench-level) were used daily to supplement natural sunlight between 06:00 and 18:00 h. Photoperiods were extended to 24:00 h with incandescent bulbs to delay flowering.

Seeds were germinated for 72 h between germination papers saturated with 0.5 mM $CaSO_4$ in a chamber maintained at 30 C and 90% RH. After their roots were dipped into a suspension of freshly cultured *Rhizobium japonicum* cells (strain USDA 110), seedlings were transplanted into 25.4-cm diameter pots (three/pot) containing Perlite mixed with approximately 250 ml crushed oyster shells. Plants were thinned to one/pot 22 days after transplanting. For the first 9 days, each pot received 250 ml of a basal, N-free nutrient solution (16) twice daily. After day 9, the pots were flushed with tap water twice daily (09:00 and 15:00 h) and nutrient solution (400 ml) was applied after the second flushing. Until day 36, all plants received the N-free nutrient solution. After day 36, one group of plants received daily applications of a modified nutrient solution containing 20 mM KNO_3, and the remaining plants received a modified N-free solution containing 10 mM K_2SO_4 to equalize the K^+ concentration to that of the KNO_3 solution. These treatments were imposed to obtain two types of plants: (*a*) plants totally dependent upon N_2 fixation, and (*b*) plants dependent upon both N_2 fixation and the uptake of exogenous nitrate.

C_2H_2 treatments were imposed on 52-day-old plants to inhibit N_2 fixation. The pots containing the root systems of intact plants were sealed in Saran bags which were fitted with perforated plastic tubes that served as inlet and outlet ports. The tubes were embedded 7 to 10 cm below the surface of the Perlite. The sealed pots were placed in a water bath at approximately 28 C. The experiment was conducted between 07:00 and 19:00 h in an unshaded greenhouse supplemented with metal halide lamps (400 μE m^{-2} s^{-1} PAR at bench-level). Approximately 1000 ml C_2H_2 were added to each sealed system for three replicate plants of each N treatment (six experimental units). The sealed systems were connected to circulating pumps (approximate flow rate, 3 liters/min), and the air in each system was mixed for 20 min. The plants were exposed to C_2H_2 for two adjacent 5.5-h periods. These periods were separated by about 30 to 45 min, during which the pots were degassed for 20 min, and fresh C_2H_2 was added for the second exposure period. Immediately following the initial 20-min mixing period and preceding the gas change at 5.5 h, gas samples were removed and measured for C_2H_2 with a Carle 311 H gas chromatograph[4] equipped with a flame ionization detector and a column of Porapak N (182 × 0.32 cm). The average C_2H_2 concentration was 0.10 ± 0.03 atm. Three replicate plants of each N treatment were sealed in the same manner as the treated plants to serve as controls. Mixing and degassing procedures were identical to those imposed on the treated plants, but no C_2H_2 was injected.

Following the 11-h treatment period, the Saran bags were removed. Each pot was saturated with tap water and supplied with 400 ml of the appropriate nutrient solution. The plants then were cut below the cotyledonary node with a razor blade, and the exuded sap was collected with capillary pipettes. The first drops of fluid to appear were blotted with a tissue and discarded. Sap was collected for 20 min and kept on ice until transfer to a freezer (−18 C) for storage.

Amino acids and ureides (allantoin and allantoic acid) in the sap samples were separated and quantified using a Durrum Chemical Corp. microbore amino acid analyzer and appropriate standards as described previously (16). Nitrate-N in the sap of the KNO_3-treated plants was determined by a manual modification of the method of Lowe and Hamilton (13). Total N in the sap samples was determined by a summation of the N in the amino acid, ureide, and nitrate fractions. A previous report demonstrated that such a summation accounts for essentially all of the N in sap samples from similarly cultured soybeans (16).

Experiment 2: Diurnal Effects on Characteristics of Xylem Sap. A separate group of 'Davis' soybean plants was grown during the same period as the plants of experiment 1 to study diurnal effects upon xylem sap exudation rate, total N, and relative ureide content. Cultural conditions were identical to those described for experiment 1 except that all plants received the basal, N-free nutrient solution throughout the growth period and, after day 50, received nutrient solution applications after both the morning and afternoon flushings. Xylem sap was collected for 20-min periods at 4-h intervals during a 24-h period starting at 16:30 h on day 59. Three plants were sampled at each interval. Thirty min before xylem sap was collected, each pot was saturated with tap water, followed by the addition of 400 ml nutrient solution. During the experimental period, plants were supplied with water and nutrient solution according to the regular schedule. The collected sap was placed in preweighed, plastic test tubes on ice. After the collection period, sap volumes were determined gravimetrically, and the samples were stored in a freezer (−18 C) for later chemical analysis. Exudation rates were determined by dividing the volume of sap by the time of collection. Amino acids and ureides in the sap samples were separated and quantified using the amino acid analyzer (16). Total N in the sap was determined by summation of the N in the amino acid and ureide fractions.

Experiment 3: Cultivar and Strain Effects on Relative Ureide Content of Sap. To examine the influence of soybean cultivar on the relative ureide content of xylem sap, 11 soybean cultivars were grown in an unshaded greenhouse for 50 days between October and December 1979. Germination, transplanting, and inoculation procedures were as described for experiment 1, as was the application of supplemental lighting. One group of plants received a N-free nutrient solution (16) throughout growth and the remaining plants received a modified solution containing 20 mM KNO_3. Nutrient solution and water were supplied to the plants on the same daily schedule as experiment 1. Xylem sap was collected for 20-min periods between 11:00 and 13:00 h on day 50. Thirty min prior to excising the shoots, 400 ml of the appropriate nutrient solution were applied to each pot. After excision, the first drops of fluid to appear were blotted with a tissue, and all subsequent sap was collected with capillary pipettes. The sap samples (three replicates for each cultivar × N treatment combination) were stored frozen (−18 C) for later chemical analysis. Total N concentration was determined using a modified Kjeldahl procedure described previously (16). Ureide-N in the sap was determined

[4] Mention of tradenames, commercial products, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the North Carolina Agricultural Research Service and does not imply approval to the exclusion of other products that may also be suitable.

using the Young and Conway procedure (29).

To examine the influence of rhizobial strain on the relative ureide content of xylem sap, 'Ransom' soybeans were inoculated with one of four *Rhizobium japonicum* strains and grown in an unshaded greenhouse for 42 days between June and August 1979. Germination, transplanting, and inoculation procedures were as described for experiment 1. No supplemental lighting was provided. Plants received a N-free nutrient solution (16) throughout growth, and nutrient solution and water were supplied on the same daily schedule as in experiment 1. Xylem sap was collected on day 42 and analyzed as described above. Cultures of strain USDA 110 and USDA 33 were supplied by Mr. F. Munevar, Department of Soil Science, North Carolina State University, and Dr. P. P. Wong, Department of Biology, Kansas State University, respectively. Dr. D. G. Blevins, Department of Agronomy, University of Missouri, supplied cultures of strain USDA 142 and of a chlorate-resistant mutant of strain USDA 142 (USDA 142 CHLR No. 1).

Experiment 4: Calibration of Relative Ureide Content of Sap with N_2 Fixation. Germinated seed of the 'Ransom' cultivar were inoculated with *R. japonicum* strain USDA 110 and transplanted into pots (two/pot) containing Perlite as described for experiment 1. The experiment contained 60 experimental units with five N treatments, four sampling dates, and three replicates of each treatment × sampling date combination. The plants were grown under natural illumination from early September to mid-October, 1979. Photoperiods were extended to 23:00 h daily with incandescent bulbs to delay flowering. Pots designated for sampling at 42 and 49 days were thinned to one plant/pot 2 weeks after transplanting; those designated for sampling at 28 and 35 days were not thinned in order to obtain sufficient xylem sap for analysis.

Nutrient solution and deionized H_2O were applied to the plants on the same daily schedule as in experiment 1. The plants received one of five solutions in which 0, 2.5, 5, 7.5, or 10 mM KNO_3, enriched with ^{15}N (approximately 5 atom % ^{15}N), was added to the N-free solution. Potassium concentrations of all solutions were equalized to that of the 10 mM KNO_3 solution by the addition of appropriate amounts of K_2SO_4.

Xylem sap was collected and analyzed as described for experiment 3. The shoot tissue was frozen after excision. After sap collection, the root systems (including nodules) were separated from the Perlite, rinsed in deionized H_2O, and frozen. Shoot and root samples were freeze-dried, weighed, and ground, and a subsample was analyzed for total N and ^{15}N.

The samples were digested using a modified semi-micro-Kjeldahl procedure (4, 17, 19) which converts all nitrogenous compounds (including NO_3^-) to ammonium. Samples of dried plant material (75 mg) were predigested overnight at room temperature in 3.5 ml of a H_2SO_4-salicylic acid solution (1 g salicylic acid + 35 ml concentrated H_2SO_4). Next, 180 mg anhydrous Na-thiosulfate were added, and the samples were digested for 1 h at 240 C in a Technicon BD-40 digestion block. After cooling, the samples were subjected to a standard Kjeldahl digestion (16) in the presence of: 1.5 g of K_2SO_4, 0.5 ml Hg catalyst (25 g HgO + 250 ml 4 N H_2SO_4), and 0.5 ml concentrated H_2SO_4. After cooling, the digested samples were diluted to 30 ml with redistilled H_2O. Approximately 1 g Zn metal was added to amalgamate the Hg. A 20-ml aliquot was removed for the determination of total N in each sample by alkaline steam distillation followed by titration with 0.01 N K-biiodate using a methyl red-methylene blue indicator.

The NH_4^+ in each remaining 10-ml aliquot was used to determine the atom per cent ^{15}N. Each aliquot was placed in a 250-ml Erlenmeyer flask, and 5 ml 50% (w/w) NaOH was added. The flasks were sealed quickly with rubber stoppers to which were attached suspended recovery vials containing 0.5 ml 0.5 N HCl. Each stopper was equipped with an outlet port attached to a two-way plastic stopcock. Through this port, the flasks were evacuated with a hand-operated vacuum pump to about 20 cm Hg vacuum. The contents were gently mixed, and the flasks were incubated in an oven at 70 C for 48 h to allow diffusion of the generated NH_3 to the recovery vial. After incubation, the contents of the recovery vials were rinsed into disposable 12- × 75-mm borosilicate glass test tubes with redistilled H_2O. The samples were taken to dryness in a heating block set at 97 C. Determinations of ^{15}N were made with a CEC 21-620 mass spectrometer after converting the NH_4^+ to N_2 by reaction with NaOBr using a freeze-layer procedure (27).

Samples of each nitrate-containing nutrient solution were collected on days 28, 37, and 44 for determination of ^{15}N. The procedure of Volk *et al.* (28) was used in which nitrate is reduced to NO by sonication with Hg in 18 n H_2SO_4 and the relative ^{14}NO and ^{15}NO concentrations are determined mass spectrometrically. The average atom per cent excess ^{15}N of the nitrate-containing nutrient solutions was 4.635 ± 0.031.

The contribution of the nutrient solution to the overall N input of a given plant tissue was calculated according to the formula:

$$\left[\frac{\text{atom per cent excess } ^{15}\text{N in tissue}}{\text{atom per cent excess } ^{15}\text{N in nutrient}}\right] \times \text{total N in tissue}$$

The atom per cent excess ^{15}N value of a given tissue of the nitrate-treated plants was calculated by subtracting the average atom % ^{15}N value measured for each tissue type of three replicate "zero-N" plants at the same sampling data from the measured atom per cent ^{15}N value of given tissue. The contribution of N_2 fixation to the overall N input of a given plant was calculated according to the formula:

[Whole plant total N] − [root N from nutrient
+ shoot N from nutrient + N from seed]

Using the same seed lot from which the experimental material was drawn, average values of dry weight per seed and per cent N (by the total N procedure already described) were 128.5 ± 20.7 mg and 6.45 ± 0.08%, respectively. An average value for the N input from the seed (0.592 mmol N) was calculated and used in the above calculation. The overall N input of a given plant was calculated by subtracting this estimate of the seed N from the whole plant total N.

The foregoing analysis for determining the N input from N_2 fixation assumes any N from sources other than the labeled nitrate, N_2 fixation and the seed to be negligible. Possible sources of N contamination were the reagents used for the nutrient solutions and the deionized H_2O. An average N concentration of 0.24 ± 0.10 mM was determined from total N analysis of samples of the "zero N" nutrient solutions. Total N determinations of samples of the deionized H_2O used in the experiment yielded similar values, thus implicating the deionized H_2O as the source of contamination. Because nitrate and ammonium were not detectable in any of the samples, the form of this N is assumed to be organic. The tap water used to generate the deionized H_2O contained detectable nitrate, and it seems plausible that the N in the deionized H_2O came from the ion-exchange resins used to deionize the water. Because of the uncertainty concerning the availability of the contaminating N in the deionized H_2O, it seems most prudent to consider the estimates of N_2 fixation in this experiment as maximal values.

RESULTS AND DISCUSSION

Inhibition of N_2 fixation by an 11-h exposure of the root systems to C_2H_2 caused a significant decrease in the total N concentration of xylem sap from soybean plants totally dependent upon N_2 fixation (Table I). C_2H_2 treatment of plants dependent upon both N_2 fixation and the uptake of exogenous nitrate caused no such

Table I. *Effect of 11-Hour C_2H_2 Exposure on Total N Concentration and Relative Ureide Content of Xylem Sap*

Each value represents the mean ± SE of three replicates.

N and C_2H_2 Treatments	Total N	Ureide-N
	μg/ml	*% of Total N*
20 mM NO_3 control	216 ± 146	37 ± 11
20 mM NO_3 + 11 h C_2H_2	215 ± 63	12 ± 2
N-free control	366 ± 233	89 ± 2
N-free + 11 h C_2H_2	24 ± 1	52 ± 9

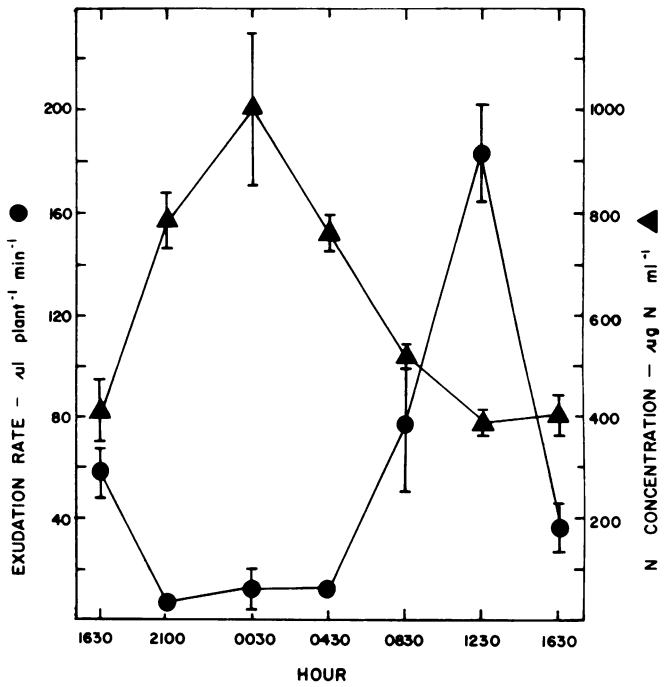

FIG. 1. Diurnal patterns of xylem sap exudation rate and total N concentration. Each value represents the mean of three replicates. Vertical bars represent ± SE of the means.

decrease in the total N concentration of xylem sap. However, C_2H_2 treatment caused a significant decrease in the relative ureide content of xylem sap in both types of plants. These results demonstrated that the relative ureide content of xylem sap is sensitive to inhibition of N_2 fixation and provided further support for a connection between the presence of ureides in the xylem and the occurrence of N_2 fixation in soybeans.

The rate of exudation of xylem sap in plants totally dependent upon N_2 fixation displayed marked diurnal variations (Fig. 1). Exudation rates were uniformly low during dark hours and began to increase during the light period to a maximum around 12:00 noon, after which the rates decreased. The total N concentration of the sap varied in an approximately inverse fashion to the variations in exudation rates. Even though there were marked diurnal effects upon the N concentration and exudation rate of xylem sap, only small diurnal variations in the relative distribution of N compounds in the xylem sap were observed (Fig. 2). The total relative ureide content (allantoin and allantoic acid) varied between 82 and 90% of the total sap N. This small variation was not correlated with the variations in N concentration and exudation rate of xylem sap displayed in Figure 1. These results demonstrated that variations in the N concentration and exudation rate of xylem sap do not greatly affect the relative ureide content of xylem sap collected during the first 20 min after decapitation.

The relative ureide contents of xylem sap from plants of 11 soybean cultivars grown under similar cultural conditions were

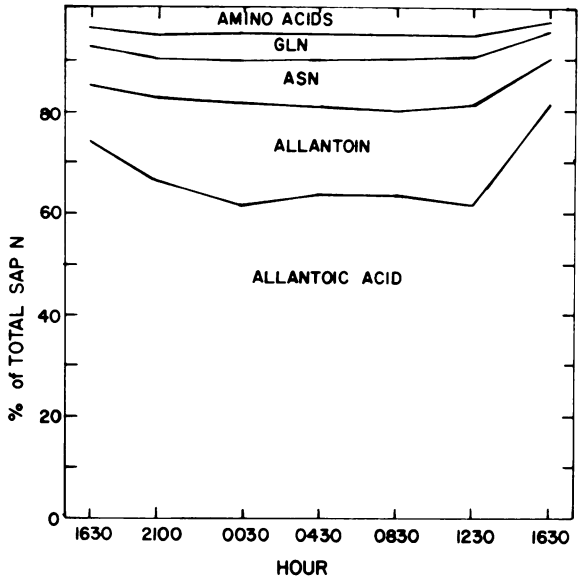

FIG. 2. Diurnal pattern of the distribution of nitrogenous compounds in xylem sap.

not significantly different (Table II). Five of the cultivars are northern United States cultivars ('Lincoln,' 'Illini,' 'Dunfield,' 'Mukden,' and 'Williams'). 'Williams' is a recently developed cultivar, and the others are cultivars released before 1950 (14). The other six are southern cultivars. 'Arksoy' was released before 1950, and the remaining five were released after 1950. Ureides represented an overall average 77.4% of the total N in the sap from plants of the 11 cultivars which were supplied a N-free nutrient solution throughout growth. Plants supplied 20 mM nitrate throughout growth had much poorer nodulation, and ureides only represented an average 7.9% of the total N in the sap. Relative ureide contents of xylem sap among the cultivars within N treatments were not significantly different at the 5% level of probability, as indicated by an analysis of variance. Under conditions of total dependency upon N_2 fixation, no significant differences were noted among the relative ureide contents of sap from 42-day-old 'Ransom' soybean plants inoculated with four different rhizobial strains (USDA 110, 84.5 ± 10.7%; USDA 33, 77.2 ± 10.3%; USDA 142 (WT), 86.8 ± 6.8%; USDA 142 (CHLR No. 1), 84.4 ± 3.3%). These results provided little evidence for soybean cultivar or nodulating strain effects upon the relative ureide content of xylem sap, thus suggesting that the use of the relative ureide content of sap as an indicator of N_2 fixation in soybeans may have widespread applicability. A greater number of cultivar and strain combinations needs to be examined under conditions of varying dependence upon N_2 fixation to characterize more thoroughly any cultivar and strain effects that may exist.

Experiment 4 was designed to calibrate relative ureide levels in xylem sap with changes in the contribution of N_2 fixation to the total N input of soybean plants of one variety ('Ransom') inoculated with one rhizobial strain (USDA 110). The applied N treatments produced plants with a spectrum of dependency upon N_2 fixation (Fig. 3). With increasing levels of exogenous nitrate, the relative contribution of N_2 fixation to the total N input decreased. Plants supplied with intermediate exogenous nitrate levels (2.5, 5, and 7.5 mM) were increasingly dependent upon N_2 fixation with age. Patterns for the relative ureide content in the xylem sap of these plants were similar (Fig. 4). As exogenous nitrate increased, relative ureide contents of sap decreased, and plants supplied with the intermediate nitrate levels had increasing relative ureide contents in their sap as they aged.

The relative ureide content of xylem sap for each of the 15

Table II. *Influence of N Source and Cultivar on Total N Concentration and Relative Ureide Content of Xylem Sap and on Nodule Dry Weight of 11 Soybean Cultivars*

Cultivars were sampled 50 days after transplanting. Each value is a mean of three replicates.

Cultivar	N Treatment					
	Zero Nitrogen			20 mM Nitrate		
	Total N	Ureide-N	Nodule Dry Wt	Total N	Ureide-N	Nodule Dry Wt
	μg/ml	*% total N*	*g/plant*	*μg/ml*	*% total N*	*g/plant*
Lincoln	405.6	79.8	1.43	357.5	11.0	0.15
Illini	336.7	80.7	0.78	314.2	9.5	0.07
Dunfield	241.8	77.9	1.17	294.2	6.8	0.04
Mukden	352.6	70.6	0.83	271.3	6.9	0.02
Arksoy	413.4	78.3	1.40	307.4	7.0	0.06
Williams	309.6	72.5	1.04	300.1	9.9	0.22
Centennial	287.4	77.9	1.22	311.1	5.3	0.08
Braxton	322.9	78.2	1.30	252.3	7.3	0.00
Essex	330.3	73.2	0.54	374.3	6.2	0.04
Tracey	443.8	80.1	1.28	330.1	9.0	0.18
Forrest	298.3	82.5	1.05	292.6	7.8	0.10

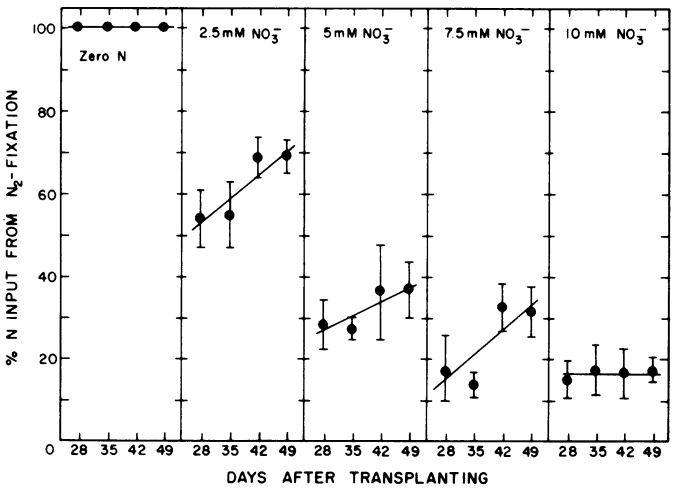

FIG. 3. The influence of exogenous nitrate level and sampling date on the relative N input from N_2 fixation in 'Ransom' soybeans. Each point represents the mean of three replicates. Vertical bars represent ± SE of the means.

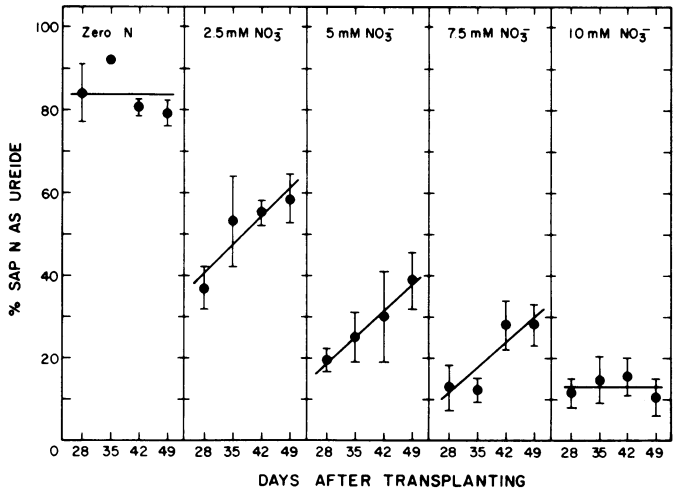

FIG. 4. The influence of exogenous nitrate level and sampling date on the relative ureide content of xylem sap from 'Ransom' soybeans. Each point represents the mean of three replicates. Vertical bars represent ± SE of the means.

individuals at each sampling date was regressed against the relative contribution of N_2 fixation to the overall N input. Good fits to linear models were obtained for each of the four regressions (Fig. 5). Statistical tests for the homogeneity of *y* intercepts and slopes (24) indicated that the *y* intercepts were not significantly different but that the slopes were statistically different at the 5% level of probability. These differences were small, however, and, when the 60 points were used to estimate a single regression, a very good fit to a linear model was obtained. These results demonstrated that the relative ureide content of xylem sap provided a reliable indicator of the relative contribution of N_2 fixation to the N input of 'Ransom' soybeans nodulated with *R. japonicum* strain USDA 110 during vegetative growth in a greenhouse.

Ohyama and Kumazawa (20) have shown that small quantities of N from exogenously supplied ^{15}N-labeled nitrate are incorporated into ureides. In experiment 4, this contribution of nitrate to the root ureide pool and subsequently to ureides in the xylem was also apparently small. This is evidenced by the extrapolated *y* intercepts of the regressions in Figure 5, which are all close to zero. However, our experiment did not examine xylem sap from plants whose relative N input from N_2 fixation was less than 10%. Small but measurable amounts of ureides have been observed in xylem sap from non-nodulating soybeans (11) and from nodulating soybeans which were noninoculated and supplied exogenous nitrate (16). These observations suggest that the linear relationships described in Figure 5 may not be applicable to soybean plants whose relative N_2 fixation rates are less than 10%.

Ishisuka (11) and Matsumoto *et al.* (15) have shown that the ureide content of xylem sap is not constant with time after decapitation and that the ureide content of sap from non-nodulated soybeans increases to the level of nodulated plants within 3 to 5 days after decapitation. Because of this phenomenon, it is important to emphasize that any use of the relative ureide content of sap to estimate N_2 fixation would require short collection periods when the relative distribution of nitrogenous compounds in xylem sap changes very little (16).

In general, the results from the experiments reported here encourage further evaluation of the use of the relative ureide content of xylem sap as an indicator of N_2 fixation in soybean plants. Additional greenhouse experiments must be conducted to

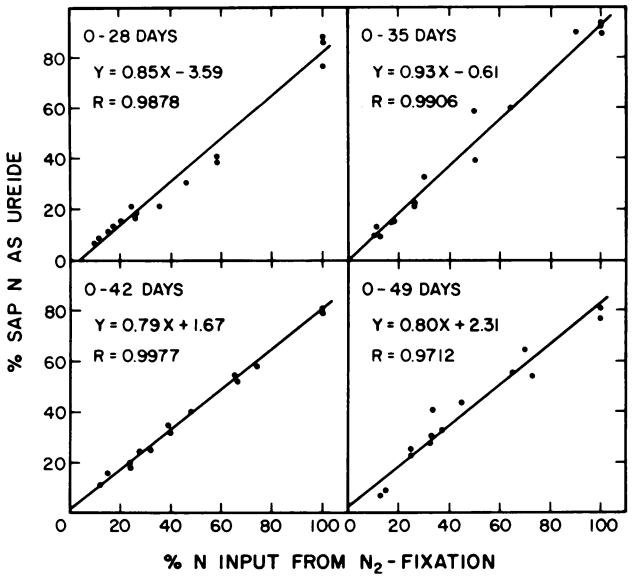

FIG. 5. Relationship between the relative ureide content of xylem sap and the relative N input from N_2 fixation in 'Ransom' soybeans at four sampling dates.

determine how the relationship between the relative ureide content of xylem sap and N_2 fixation as described in Figure 5 is influenced by variability in cultural conditions, stage of development (Experiment 4 only examined plants during vegetative growth), and rhizobial strain-host plant genotype combinations. If the relationship is demonstrated to be essentially constant with respect to these factors, it should be feasible to use seasonal patterns of relative ureide contents in collected xylem sap and total N accumulation in the tissue to estimate seasonal N_2 fixation by a soybean crop in the field.

Acknowledgments—The authors are grateful to V. G. Flynt, L. E. Cobb, and P. V. Windsor for their excellent technical assistance.

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