Plant Physiol. (1979) 64, 411–416
0032-0889/79/64/0411/06/$00.50/0

Transport of Nitrogen in the Xylem of Soybean Plants[1]

Received for publication February 6, 1979 and in revised form April 25, 1979

PETER R. MCCLURE AND DANIEL W. ISRAEL
Department of Soil Science and United States Department of Agriculture, Science and Education Administration, Agricultural Research, Department of Soil Science, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

Experiments were conducted to characterize the distribution of N compounds in the xylem sap of nodulated and nonnodulated soybean plants through development and to determine the effects of exogenous N on the distribution of N compounds in the xylem. Xylem sap was collected from nodulated and nonnodulated greenhouse-grown soybean plants (*Glycine max* [L.] Merr. "Ransom") from the vegetative phase to the pod-filling phase. The sum of the nitrogen in the amino acid, nitrate, ureide (allantoic acid and allantoin), and ammonium fractions of the sap from both types of plants agreed closely with total N as assayed by a Kjeldahl technique. Sap from nodulated plants supplied with N-free nutrient solution contained seasonal averages of 78 and 20% of the total N as ureide-N and amino acid-N, respectively. Sap from nonnodulated plants supplied with a 20 millimolar KNO_3 nutrient solution contained seasonal averages of 6, 36, and 58% of total N as ureide-N, amino acid-N, and nitrate-N, respectively. Allantoic acid was the predominant ureide in the xylem sap and asparagine was the predominant amino acid. When well nodulated plants were supplied with 20 millimolar KNO_3, beginning at 65 days, C_2H_2 reduction (N_2 fixation) decreased relative to nontreated plants and there was a concomitant decrease in the ureide content of the sap. A positive correlation (r = 0.89) was found between the ureide levels in xylem sap and nodule dry weights when either exogenous nitrate-N or urea-N was supplied at 10 and 20 millimolar concentrations to inoculated plants. The results demonstrate that ureides play a dominant role in N transport in nodulated soybeans and that the synthesis of ureides is largely dependent upon nodulation and N_2 fixation.

The form in which N is transported from the assimilatory root to the shoot has been found to vary widely among higher plants. Nitrate, amino acids, amides, and ureides all have been implicated as principal forms of N in the xylem sap of various plants (2, 19–21). Work from a number of laboratories in Japan has led to the hypothesis that the ureides, allantoin and allantoic acid, are important in the translocation of N in nodulated soybean plants. Large quantities of allantoin and allantoic acid were accumulated in the soluble N fraction of nodulated soybean plants, whereas very little ureide-N was accumulated in a nonnodulating soybean variety (13). It was also demonstrated (15) that treatment of soybean plants with exogenous N (NO_3^- and urea) caused a decrease in nodule weight and a decrease in ureide accumulation. Positive correlations between nodule weight and accumulation of allantoin and allantoic acid have suggested that the greater portion of ureide synthesis occurs in the nodules or that nodules stimulate the production of ureides in the root tissue (7, 15). Evidence that ureide synthesis occurs in the nodules was provided by the observation that the incorporation of ^{15}N from $^{15}N_2$ into allantoin and allantoic acid in nodules was higher than that in the basal portion of roots (14). Further, nodules of 4-week-old soybean plants have been reported to have significant activities of xanthine oxidase and uricase, which catalyze the oxidative decomposition of xanthine into allantoin, whereas roots and stems have only low activity levels of these enzymes (25).

It is apparent that allantoin and allantoic acid play a central role in the N metabolism of nodulated soybean plants, and thus possibly are important in the translocation of N from nodulated roots to the shoot. Matsumoto *et al.* (12) and Ishisuka (10) have reported that soybean xylem sap contained considerable amounts of allantoin and allantoic acid. Streeter (23) reported that asparagine was the principal N compound in the xylem sap of field-grown nodulated soybean plants. However, no total N analysis was performed. Recent reanalysis (24) of the sap from this study (which had been stored frozen) showed that ureide-N concentrations during reproductive development were two to six times greater than amino acid plus nitrate-N concentrations. The present studies were designed to: (*a*) characterize the distribution of all nitrogenous compounds in the xylem sap of nodulated and nonnodulated soybean plants throughout development; and (*b*) determine the effect of exogenous N upon the distribution of nitrogenous compounds in the xylem.

MATERIALS AND METHODS

PLANT CULTURE

Experiment 1. Soybean seeds (*Glycine max* [L.] Merr., "Ransom") were germinated in paper towels saturated with 0.5 mM $CaSO_4$ in a chamber maintained at 30 C and 90% RH. Three days after imbibition (May 20, 1977) seedlings (three per pot) were transplanted into pots (25.4-cm diameter) containing Perlite mixed with ≃ 250 ml of crushed oyster shells per pot. Plants were grown in an unshaded greenhouse from the time of transplanting until final harvest in mid-September. Evaporative cooling of the greenhouse kept the maximum day temperatures below 35 C and the plants displayed no apparent heat damage. The roots of seedlings for the inoculated treatments were dipped into a suspension of freshly cultured *Rhizobium japonicum* cells (strain USDA 110) just before transplanting. Two weeks after transplanting the seedlings were thinned to two per pot. For the first 9 days, 250 ml of nutrient solution (described below) was applied to each pot twice daily. From day 9 to the 8th week, the pots were flushed with tap water twice daily (9:00 AM and 4:00 PM), and 400 ml of nutrient solution was applied to each pot after the second flushing. From the 8th week until the last sampling date (126 days), the pots were flushed with tap water three times daily (9:00 AM, noon, and 4:00 PM) and 400 ml of nutrient solution was applied to each pot after the first

[1] Cooperative investigations of the United States Department of Agriculture, Science and Education Administration, Agricultural Research and the North Carolina Agricultural Research Service, Raleigh, North Carolina. Paper No. 5936 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 PRM.

and third flushings. Because Perlite in each pot had a water-holding capacity of approximately 2 liters, the 400-ml additions of nutrient solutions were diluted about 5-fold.

The basal N-free nutrient solution contained 7 mM $CaSO_4 \cdot 2H_2O$, 17.8 μM Fe-EDTA, 1.0 mM K_2SO_4, 0.25 mM KH_2PO_4, 0.625 mM K_2HPO_4, 2.0 mM $MgSO_4 \cdot 7H_2O$ and micronutrients as described by Ahmed and Evans (1). Three nutrient treatments were imposed. Noninoculated plants received the basal nutrient solution containing 20 mM KNO_3. One group of inoculated plants received, throughout growth, the basal nutrient solution to which 10 mM K_2SO_4 was added to equalize the K^+ concentration to that of KNO_3 solution. Another group of inoculated plants received the modified N-free solution through the first 65 days but was then supplied the 20 mM KNO_3 solution through the remainder of the experiment. The initial pH of all solutions was 6.8. Three replicates of each treatment were grown for each of eight sampling dates with pots placed in randomized complete blocks.

Experiment 2. A separate group of "Ransom" soybean plants was grown at the same time to examine more closely the effects of exogenous N upon the distribution of N compounds in the xylem sap (see Table III). All plants were inoculated at transplanting with *R. japonicum* strain USDA 110 and received the basal nutrient solution containing no N, 10 and 20 mM nitrate-N, and 10 and 20 mM urea-N. Potassium concentrations of all treatment solutions were equalized to that of the 20 mM KNO_3 treatment solution by addition of appropriate amounts of K_2SO_4. The initial pH of all solutions was 6.8. Two weeks after transplanting, seedlings were thinned to one per pot. Each treatment was replicated three times, and pots were arranged in randomized complete blocks. All other cultural conditions were identical to those described for the first experiment.

Experiment 3. Another group of "Ransom" soybean plants inoculated with *R. japonicum* strain USDA 110 and supplied the basal N-free nutrient solution (pH 6.8) throughout growth was used to examine the effect of time after decapitation on exudation rate and N composition of xylem sap (see Table I). Two weeks after transplanting, seedlings were thinned to one per pot. Plants were grown in an unshaded greenhouse for a 53-day period from mid-July to early September in 1978. All other cultural conditions were identical to those described for the first experiment.

STEM SAP COLLECTION

Sap was collected between 11:00 AM and 1:00 PM on all sampling dates in all experiments to reduce diurnal variability (18). In experiments 1 and 2 collection periods from individual plants were no longer than 20 min. In experiment 3 (see Table I) sap was collected for 5-min intervals up to 20 min from three replicate groups of two plants each. Stems were cut just below the cotyledonary node with a razor blade. Sap exuding from cut surfaces was collected with capillary pipets. Samples were kept on ice until they could be transferred to a freezer (−18 C) for storage. Exudation rates ranged from 0.01 to 0.5 ml per plant per min.

STEM SAP ANALYSIS

Total N of the sap was assayed with a modification of the Kjeldahl technique (8, 17) to digest all nitrogenous compounds (including NO_3^-) to NH_4^+. To freeze dried aliquots (0.2–0.3 ml) of undiluted sap, 0.4 ml of a salicylic acid-H_2SO_4 solution (1 g salicylic acid + 35 ml concentrated H_2SO_4) was added. The solutions were vortexed and allowed to stand in stoppered test tubes overnight at 25 C. Next, 18 mg of Na-thiosulfate was added and the samples were digested for 1 h in a sand bath controlled at 240 C. These steps promoted the reduction of nitrate to NH_4^+ (17). After cooling, the following reagents were added to each sample: 0.05 ml concentrated H_2SO_4; 0.02 ml Zr catalyst solution (67.5 g $ZrOSO_4 \cdot H_2SO_4 \cdot 3H_2O$ diluted to 250 ml with 0.1 N H_2SO_4); 0.02 ml Cu catalyst solution (34 g $CuSO_4 \cdot 5H_2O$ diluted to 100 ml with H_2O); and 0.18 g K_2SO_4. The samples were placed in a sand bath controlled at 240 C for 45 min. The temperature was then brought to 380C and the samples were digested for 1 h after they cleared. The digested samples were then diluted to 10 or 25 ml with redistilled H_2O. Total N as NH_4^+ in the diluted digests was then colorimetrically assayed with a hypochlorite-nitroprusside reaction (4). *A* at 625 nm was measured with a Hitachi model 100-40 spectrophotometer. Preliminary studies of the total procedure (Kjeldahl digestion and NH_4^+ detection) indicated 99.8 ± 4.9%, 101.6 ± 4.6%, and 102.1 ± 3.8% recoveries of N from standard aliquots containing between 14 and 280 μg N as nitrate, glycine, and allantoin, respectively.

Nitrate-N was determined by a manual modification of the method of Lowe and Hamilton (11) which utilizes preparations of soybean nodule bacteroids to reduce nitrate to nitrite. After addition of diazo-coupling agents, the *A* of the nitrite chromophore was read at 540 nm.

Amino acids and NH_4^+ were separated and quantified using a Durrum[2] Chemical Corporation micro-bore amino acid analyzer equipped with a column of Durrum DC-4A resin (0.32 × 25–30 cm). Compounds were eluted with five lithium buffers of nearly constant pH (about 2.8) and varying ionic strength (Durrum Pico-Buffers IVA-IVE). Detection was accomplished with ninhydrin and a 570 nm photometer. Estimated extinction coefficients of the amino acid derivatives ranged from 3.3×10^4 cm²/mol for proline to 1.0×10^6 cm²/mol for aspartic acid.

Before injection into the analyzer, sap samples were freeze-dried, resuspended in equal volumes of the first eluting solution of the system (altered to pH 2.1), and centrifuged at 15,600*g* for 5 min. Identification of amino acids and NH_4^+ in the sap samples were made by co-chromatography with standards obtained from Pierce Chemical Company.

The Durrum analyzer system was used to separate and quantify ureides (allantoin, allantoic acid, and urea) in the sap samples. Although authentic samples of these compounds (obtained from Sigma Chemical Co.) were found to be ninhydrin-negative when analyzed on thin layers of cellulose, they were quantitatively detected with ninhydrin in the Durrum analyzer system. Detection was less sensitive at 570 nm for the ureide derivatives than for those of the amino acids; extinction coefficients were 4.2×10^3 cm²/mol, 1.7×10^4 cm²/mol, and 1.3×10^4 cm²/mol for derivatives of allantoin, allantoic acid, and urea standards, respectively. Good separation was achieved among the three ureides, and they all eluted from the column well in advance of the first amino acid. We have reported the summation of the N in these compounds (*i.e.* ureide-N) in the present study because the sap samples were prepared for chromatography in a lithium buffer at pH 2.1. While authentic allantoin samples were stable in this acidic buffer, allantoic acid standards decomposed to urea (1 mol of allantoic acid yielded 2 mol of urea). The degree of decomposition was dependent upon the duration of exposure of allantoic acid to the acidic conditions. When freeze-dried aliquots of a sap sample were exposed to the pH 2.1 lithium buffer at 25 C for 10, 210, and 360 min, urea accounted for 3.8, 35.1, and 55.3% of the sum of allantoic acid-N and urea-N, respectively.

Selected sap samples were analyzed for allantoin and allantoic acid by the method of Young and Conway (26) which degrades these two compounds to urea and glyoxylic acid and colorimetrically determines the glyoxylic acid.

ACETYLENE REDUCTION ASSAYS

Nitrogen-fixation activity, as assayed by C_2H_2 reduction to C_2H_4, was measured immediately following sap collections on

[2] Mention of a trademark, proprietary 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 or vendors that may also be suitable.

days 43, 78, 85, 99, and 126, in the first experiment. Each pot containing two decapitated root systems was sealed in a Saran bag which was fitted with glass tubes that served as inlet and outlet ports. The pressure port of a circulating air pump was connected to the inlet port which had its lower end embedded 7 to 10 cm below the surface of the Perlite. The vacuum port was connected to the outlet port near the bottom of the bag. After addition of 0.1 atm of C_2H_2 (≃1,500 ml) and 5 ml of pure CH_4 (internal standard for determination of volume), the gas phase of each system was circulated continuously with an air pump at a flow rate of 3 liters/min. Gas samples (0.5 ml) were removed at 15 and 45 min for measurement of C_2H_4 and CH_4 with a Carle model 311 H gas chromatograph equipped with a flame ionization detector and a column of Porapak N (182 × 0.32 cm). Acetylene reduction activity was based on the amount of C_2H_4 formed between 15 and 45 min. Because the rate of C_2H_2 reduction by intact soybean plants has been shown to decline within 30 min of shoot removal (16), maximal values were probably not measured in the present study. However, the values should reflect relative differences in N_2-fixation capability of the plants exposed to different treatments.

RESULTS

Noninoculated plants displayed greater vegetative dry matter accumulation than inoculated plants which received no nitrate-N (Fig. 1). The inoculated plants supplied with nitrate-N after 65 days increased their dry matter accumulation to that of the noninoculated plants by the last sampling data. All plants were in full bloom by 65 days after transplanting. Pods first became evident in all treatments between 85 and 99 days, and most plants had reached the mid pod-fill stage at the last sampling date (126 days). During the experimental period, no statistically significant differences in pod dry weight accumulation were noted among the treatments.

Nodules were scarce to nonexistent on the noninoculated plants, whereas nodules were abundant on the inoculated plants supplied with N-free nutrient solution (Fig. 2). Treatment of inoculated plants with 20 mM KNO_3 after 65 days reduced accumulation of nodule dry matter resulting in 60% less nodule dry weight at the last two sampling dates relative to the untreated plants (Fig. 2).

Inoculated plants which received no nitrate-N displayed a sharp increase in C_2H_2 reduction activity after 78 days, whereas noninoculated plants showed low levels of activity (Fig. 3). Inoculated plants treated with KNO_3 displayed no increase in C_2H_2 reduction activity after 78 days (Fig. 3). The observed inhibition of nodulation and C_2H_2 reduction by nitrate was expected as it has been observed frequently (*cf.* 5).

The total N concentrations of stem sap samples were measured by two methods: (*a*) a modified Kjeldahl procedure (*cf.* "Materials and Methods"), and (*b*) a summation of the N analyzed in the nitrate, amino acid, ammonium, and ureide fractions. The mean of the ratios of the total N concentrations measured by the summation method to those measured by the Kjeldahl procedure was 0.986 ± 0.113, thus indicating that the nitrate, amino acid, ammonium, and ureide fractions contained essentially all of the N in stem sap of all treatments. This check was essential to the fulfillment of the objectives of this study. Because of this close agreement, only the results from the summation method are reported. No significant differences due to N treatments were noted in sap total N concentrations, although there was a general trend toward decreasing concentrations as the plants aged (Fig. 4). The significance of this decrease with respect to the amount of N supplied from the root to the shoot cannot be ascertained from studies of this sort.

As discussed by Pate (19) and Streeter (23), the rate of water flow and the N concentration of xylem fluid in a decapitated plant are most likely different from those in a plant with its transpirational stream intact. However, on the assumption that the rate of flow upward does not significantly alter the processes involved in deposition of substances into the xylem, analyses of bleeding sap may be used to determine the molecular compounds responsible for the transport of elements in the xylem of plants. This assumption must be viewed with some caution as indicated by an analysis of sap collected at 5-min intervals from 53-day-old nodulated soybean plants (Table I). The rate of flow upward significantly decreased after the first and second intervals. This decrease was accompanied by a general trend toward increasing relative amounts of ureide-N in the sap and decreasing relative amounts

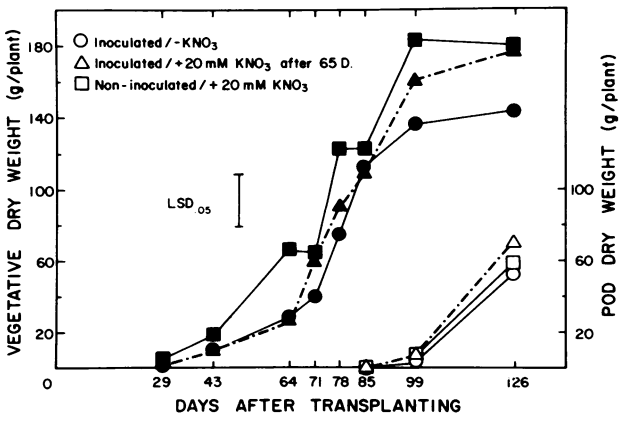

FIG. 1. Influence of inoculation and KNO_3 on vegetative and reproductive dry matter production. Each point is a mean of three replicates. (●, ■, ▲): Vegetative dry weights; (○, □, △): pod dry weights.

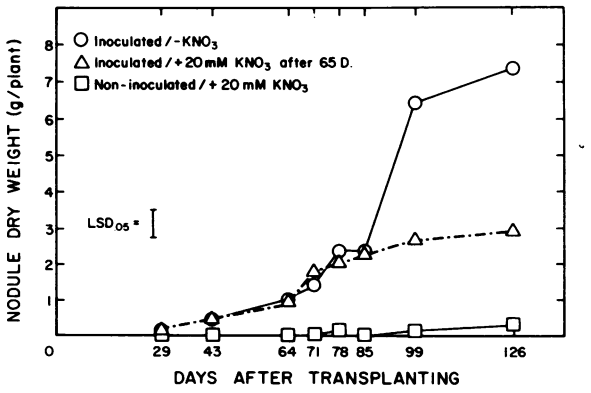

FIG. 2. Influence of inoculation and KNO_3 on nodule dry matter production. Each point is a mean of three replicates.

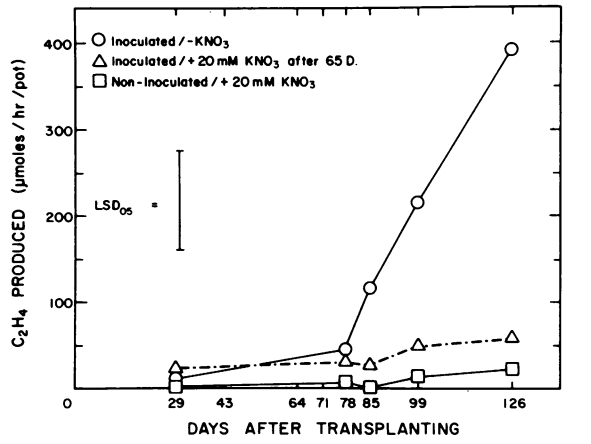

FIG. 3. Influence of inoculation and KNO_3 on C_2H_2 reduction (N_2 fixation) activity. Each point is a mean of at least two replicates.

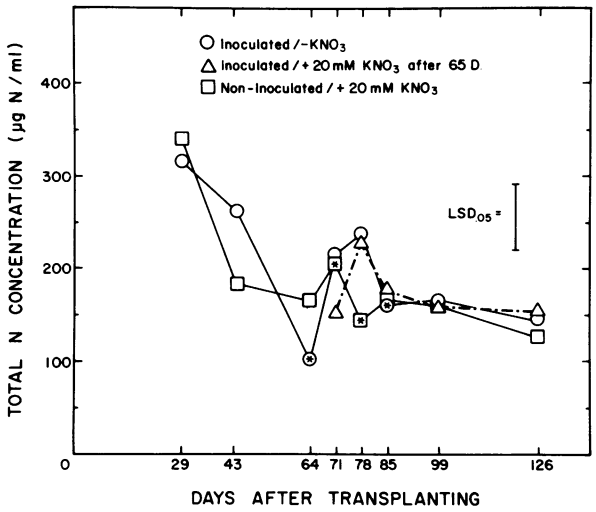

FIG. 4. Influence of inoculation and KNO_3 on total N concentration of xylem sap. Total N concentrations are the summation of the N in the nitrate, amino acid, ammonium, and ureide fractions in each sap sample. Each point is a mean of three replicates, except those designated with (*) which are means of two replicates.

Table I. *Effect of Time after Decapitation on Exudation Rate and Distribution of N Compounds in Sap from 53-Day-Old "Ransom" Soybean Plants*

Plants were in late stages of vegetative growth at time of sap collection. Each value is a mean of three replicates.

Time after Decapitation	Rates of Exudation	Ureide-N	Amide-N	α-Amino-N
min	*ml/min·plant*		*% of Total N*	
0–5	0.1339	74.0	20.5	5.3
5–10	0.0886	75.6	19.8	4.5
10–15	0.0463	79.5	16.6	3.9
15–20	0.0479	81.6	14.5	3.7
LSD (5%)	0.0247	5.1	4.8	0.9

of amide-N (from glutamine and asparagine) and α-amino-N. The small extent to which the distribution of N compounds changed in 20 min after decapitation warrants the use of the foregoing assumption in our experiments. Longer collection periods bring about drastic changes in the distribution of N compounds in soybean sap (12), rendering the assumption untenable.

The relative amount of nitrate-N in the sap of inoculated plants supplied with N-free nutrient solution was constant with development and represented only 1% of the total N (Fig. 5). The source of this nitrate was presumed to have been the tap water used to flush the pots (nitrate concentration = 44 μM). Nitrate-N represented a seasonal average of 58% of the total N in the sap of the noninoculated plants supplied with 20 mM KNO_3 (Fig. 5). While fluctuations were apparent, no statistically significant trend of decreasing or increasing percentage nitrate-N with age could be demonstrated. The relative amount of nitrate-N in the sap of inoculated plants treated with KNO_3 after 65 days increased sharply after day 71 to a level slightly lower than that of the noninoculated plants (Fig. 5).

From the observation that nitrate only represented 50 to 60% of the total N in the sap from the nitrate-grown noninoculated plants (Fig. 5) one may infer that up to 40 to 50% of the entering nitrate was reduced in the root system. However, this inference must be viewed with caution because it is conceivable that the reduced N in the sap represented N which had been originally transported to the shoot as nitrate, reduced in the shoot, translocated back to the root system via the phloem, and redeposited in the xylem in the roots.

Amino acids comprised a seasonal average of 20 and 36% of the total N in the sap of the inoculated and noninoculated plants, respectively (Fig. 6). The relative amount of amino acids in the sap of the noninoculated plants tended to increase with age from an estimated 29% at 29 days to 44% at 126 days (slope = 0.15%/day; $r = 0.64$) (Fig. 6). The relative amount of amino acids in the sap of the nitrate-treated, inoculated plants increased after day 71 to a level similar to that of the noninoculated plants (Fig. 6).

In all treatments, approximately 90% of the N in the amino acid fraction of the xylem sap could be accounted for by six amino acids (Table II). Twelve other amino acids were detected which individually represented less than 1% of the total sap N. Collectively, these amino acids contained only 2% of the total sap N (Table II). The low levels of ammonium in the sap of all treatments (Table II) indicated that the amide-N of the asparagine and glutamine was stable in our analytical procedure.

Asparagine was clearly the predominant amino acid in the xylem fluid in all treatments (Fig. 7 and Table II). Significant differences between treatments were noted in the relative amounts of asparagine. Asparagine contained an average 53% of the total amino acid-N in the sap of the inoculated plants; an average of

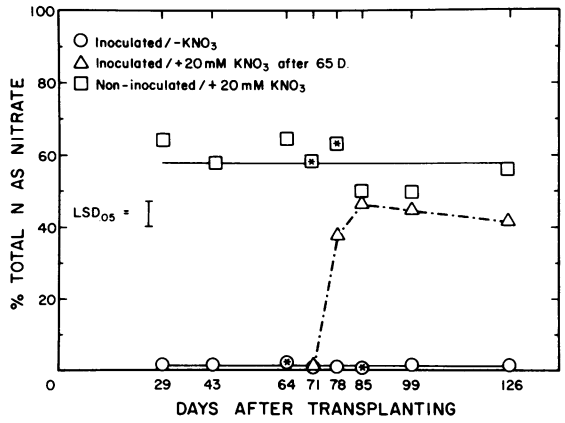

FIG. 5. Nitrate-N as per cent of total N in xylem sap. Each point designated with (*) is a mean of two replicates; all others are means of three replicates. Horizontal lines were used to represent treatment means averaged across replicates and sampling dates since linear regression coefficients were not significantly different than zero.

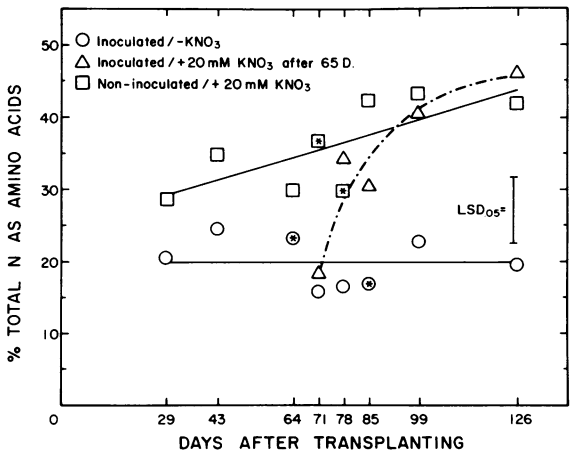

FIG. 6. Amino acid-N (α-amino and amide) as per cent of total N in xylem sap. Each point designated with (*) is a mean of two replicates; all others are means of three replicates. Horizontal lines were used to represent treatment means averaged across replicates and sampling dates when linear regression coefficients were not significantly different than zero. Data for noninoculated plants are fitted to a linear model ($y = 0.15x + 25.1$; $r = 0.64$).

Table II. *Amino Acid and Ammonium Composition of Xylem Sap*

Values are treatment means ± SE averaged across replicates and sampling dates. Amino acids in "others" category individually represented less than 1% of the total N, and included glycine, alanine, valine, cysteine, methionine, leucine, isoleucine, tyrosine, phenylalanine, glutamic acid, threonine, and serine.

Amino Acid	% of Total N as Specific Amino Acids and Ammonium		
	Inoculated/ $-KNO_3$[a]	Inoculated/ $+KNO_3$ after 65 days[b]	Noninoculated/ $+KNO_3$[a]
Asn	10.5 ± 2.7	25.0 ± 9.6	26.9 ± 6.7
Gln	3.3 ± 1.4	2.8 ± 0.9	2.4 ± 1.1
Asp	1.1 ± 0.8	1.5 ± 1.0	2.0 ± 1.5
Arg	1.2 ± 0.5	0.8 ± 0.3	0.7 ± 0.3
Lys	0.6 ± 0.3	0.5 ± 0.2	0.6 ± 0.2
His	0.8 ± 0.3	1.1 ± 0.3	1.2 ± 0.2
Others	2.2	2.3	2.1
Total amino acids	19.7 ± 4.5	34.0 ± 10.4	35.9 ± 7.1
NH_4^+	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.3

[a] Values are averaged over eight sampling dates.
[b] Values are averaged over five sampling dates.

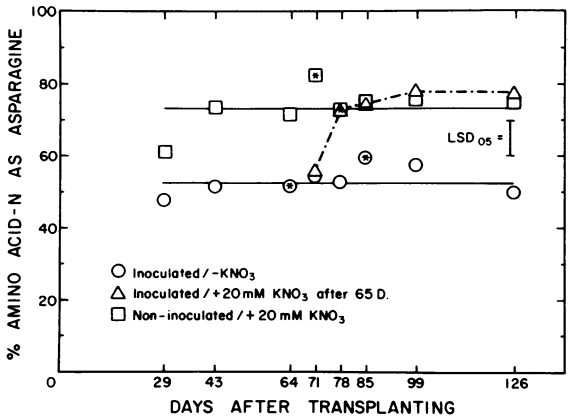

FIG. 7. Asparagine-N as per cent of total N in xylem sap. Each point designated with (*) is a mean of two replicates; all others are means of three replicates. Horizontal lines were used as in previous figures.

74% was calculated for the noninoculated plants (Fig. 7). After day 71, the relative amount of asparagine in the sap of the nitrate-treated, inoculated plants increased to the level characteristic of the noninoculated plants (Fig. 7).

Ureides, clearly the predominant nitrogenous compounds in the sap of the inoculated plants throughout the experimental period, were conspicuously scarce in the sap of the noninoculated plants (Fig. 8). Ureides represented 78 and 6% of the total N in the sap of the inoculated and noninoculated plants, respectively. A dramatic decrease was noted after 71 days in the level of ureides in the sap of the nitrate-treated, inoculated plants (Fig. 8). These results demonstrated that nitrate and amino acids were the dominant N forms in the xylem sap of nitrate-grown plants, whereas ureides were the dominant N forms in the sap of plants dependent upon N_2 fixation.

Within the ureide fraction of the sap from the inoculated plants, allantoic acid was the predominant compound, representing 57.2 ± 9.7% of the ureide-N. Allantoin and urea represented 20.0 ± 6.9 and 22.8 ± 9.0% of the ureide-N, respectively. These values underestimate the importance of allantoic acid (and overestimate the importance of urea), because the sap samples were prepared for chromatography in a lithium buffer at pH 2.1 and the time of exposure to the lithium buffer was not standardized (*cf.* "Materials and Methods"). The estimated N in allantoin and allantoic acid of selected samples as assayed by the Young and Conway method (26) was in close agreement with the N in allantoin, allantoic acid, and urea as assayed by the amino acid analyzer method. As determined by the Young and Conway method, allantoic acid represented approximately 80% of the total ureide-N in these selected sap samples. Most of the urea in the sap, as assayed by the amino acid analyzer method, was apparently an artifact of the procedure.

In experiment 2, xylem sap was collected from 76-day-old "Ransom" soybean plants which were nodulated by *R. japonicum* strain USDA 110 and had been supplied various levels of exogenous urea and nitrate. A striking decrease in the per cent of total sap N as ureides was noted as levels of both exogenous N sources increased (Table III). A corresponding increase in the per cent of total sap N as amino acids and nitrate accompanied the decrease in ureides. These results demonstrated that ureides were most important in N transport under nodulated conditions in the absence of exogenous, combined N and that amino acids or nitrate play an increasingly important role as levels of exogenous N are raised.

A positive correlation ($r = 0.89$) was noted between the ureide levels expressed as a per cent of total N in xylem sap and nodule dry weights (Table III), thus suggesting that nodules were responsible for a large portion of ureide synthesis. The presence of ureides in the sap of noninoculated, nitrate-treated plants (Fig. 8) indicated that a limited amount of ureide synthesis occurred in the root independently of the presence of nodules.

DISCUSSION

The results from these experiments demonstrated that the two systems of N assimilation in the soybean plant are reflected in the distribution of N compounds in the xylem sap. With the assimi-

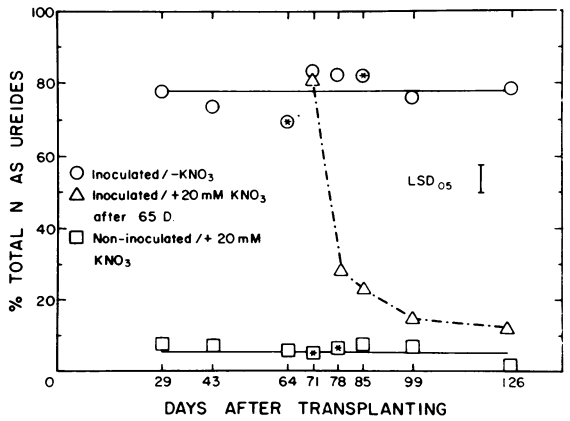

FIG. 8. Ureide-N as per cent of total N in xylem sap. Each point designated with (*) is a mean of two replicates; all others are means of three replicates. Horizontal lines were used as in previous figures.

Table III. *N Composition of Xylem Sap from 76-Day-Old "Ransom" Soybeans as Related to Source of N and Nodule Dry Weight*

Plants were in full flower at the time of sap collection. Each value is a mean of three replicates.

Treatment	Total N	Ureide-N	Amino Acid-N	Nitrate-N	NH_4^+-N	Nodule Dry Weight
	μg/ml	% of Total N				% of zero nitrogen treatment
Zero nitrogen	187.4	83.0	16.3	0.4	0.2	100.0
10 mM nitrate-N	134.1	47.5	26.4	25.8	0.3	39.2
20 mM nitrate-N	138.0	15.0	37.6	47.0	0.4	12.9
10 mM urea-N	173.8	49.4	48.0	2.4	0.2	44.1
20 mM urea-N	192.9	39.3	56.8	3.5	0.3	29.0
LSD (5%)	34.5	15.5	13.4	7.3	0.2	28.8

lation of exogenous combined N, amino acids and nitrate were the predominant forms of N transport in the xylem. With the assimilation of N_2, ureides were the major agents for xylary N transport. The ratio of amino acids and nitrate to ureides in the xylem varied depending upon the extent to which external, combined N was supplied (Table III).

Allantoin and allantoic acid are produced in bacteria (3), yeasts (22), and other microorganisms via the oxidative decomposition of purines. This pathway operates in nodulated soybeans (6). Upon treatment with allopurinol, an inhibitor of xanthine oxidase, allantoin and allantoic acid content of nodules and stems of 35-day-old soybean plants sharply decreased. A correspondingly sharp increase occurred in the xanthine content of the nodules but not of the roots or stems. From these results it was concluded (6) that allantoin and allantoic acid are formed within the nodules of nodulated soybeans through purine decomposition.

Our data clearly indicate that the major portion of ureide synthesis is associated with the presence of nodules (Fig. 8 and Table III), but the presence of low levels of ureides in the xylem sap from the noninoculated plants (Fig. 8) suggests that some ureide production also occurs in the absence of nodules. Some uncertainty must accompany this interpretation because our growing conditions were not sterile and some nodules were found at various sampling dates on the noninoculated plants (Fig. 2). Additional evidence that some ureide production occurs in the absence of nodules has been provided by the observation (13) of measurable amounts of allantoin and allantoic acid in a nonnodulating soybean variety, although the amounts were very small compared to the amounts in a nodulating variety.

While nodules may or may not be the exclusive site of ureide production, it is certain that most ureide synthesis is associated with nodules and that allantoin and allantoic acid play a central role in N transport and metabolism of nodulated, N_2-fixing soybean plants. Ureides recently have been shown to play a similar role in nodulated cowpea plants (*Vigna unguiculata* [L.] Walp.) (9). Further investigation is needed to determine whether the "cytosol" or bacteroid fraction of the nodule is responsible for ureide synthesis and to determine the role that ureides may play in regulation of N_2 fixation.

The positive correlation between ureide content of sap and nodule dry weight (Table III) suggests that the relative ureide content of xylem sap may be used as an index of the effective nodulation of field-grown plants and the relative contribution of N_2 fixation to the total input of plant N. Further support for this suggestion was provided by experiment 1. The plants totally dependent on N_2 fixation displayed dramatic increases in nodule mass and C_2H_2 reduction activity after 65 days, whereas only small changes in these characteristics were noted in the nitrate-supplied inoculated plants during the same period (Figs. 2 and 3). This comparison indicates that the relative contribution of N_2 fixation to the total input of plant N was greatly diminished in nodulated plants that were given a continuous supply of nitrate after 65 days. Because the relative abundance of ureide-N in the xylem sap of the nitrate-treated plants decreased greatly during this period (Fig. 8), it seems reasonable to suggest that the percent ureide-N in xylem sap may be used as an indicator of the relative contribution of N_2 fixation to the total input of plant N. In addition, the relative ureide content of the sap from the nitrate-treated nodulated plants never decreased to the level of the sap from the noninoculated nitrate-treated plants (Fig. 8). Further work is needed to calibrate the relative ureide content of xylem sap with the relative contribution of N_2 fixation to the total input of plant N.

Acknowledgments—We would like to thank Dr. B. Klubek for providing fresh cultures of *R. japonicum* as inoculum and Drs. W. A. Jackson, C. D. Raper, C. T. Mackown, R. J. Volk, R. C. Fites, and R. J. Fellows for constructive criticism of the manuscript during preparation. We also thank Ms. Virginia Flynt for expert technical assistance.

LITERATURE CITED

1. Ahmed S, HJ Evans 1960 Cobalt: a micronutrient element for the growth of soybean plants under symbiotic conditions. Soil Sci 90: 205–210
2. Bollard EG 1957 Translocation of organic nitrogen in the xylem. Aust J Biol Sci 10: 292–301
3. Bongaerts GPA, GD Vogels 1976 Uric acid degradation by *Bacillus fastidiosus* strains. J Bacteriol 125: 689–697
4. Cataldo DA, LE Schrader, VL Youngs 1974 Analysis by digestion and colorimetric assay of total nitrogen in plant tissues high in nitrate. Crop Sci 14: 854–856
5. de Mooy CJ, J Pesek, E Spaldon 1973 Mineral nutrition. *In* BE Caldwell, ed, Soybeans: Improvement, Production, and Uses, Chap 4. Amer Soc of Agronomy, Inc, Madison, pp 267–352
6. Fujihara S, M Yamaguchi 1978 Effects of allopurinol [4-hydroxy pyrazolo (3,4-d)pyrimidine] on the metabolism of allantoin in soybean plants. Plant Physiol 62: 134–138
7. Fujihara S, M Yamaguchi 1978 Probable site of allantoin formation in nodulating soybean plants. Phytochemistry 17: 1239–1243
8. Glowa W 1974 Zirconium dioxide, a new catalyst in the Kjeldahl method for total nitrogen determination. JAOAC 57: 1228–1230
9. 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
10. Ishisuka J 1977 Function of symbiotically fixed nitrogen for grain production in soybean. Proc Int Sem Soil Environment Fertility Management in Intense Agriculture, pp 618–624
11. Lowe RH, JL Hamilton 1967 Rapid method for determination of nitrate in plant and soil extracts. J Agric Food Chem 15: 359–361
12. 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 47: 463–469
13. Matsumoto T, M Yatazawa, Y Yamamoto 1977 Distribution and change in contents of allantoin and allantoic acid in developing nodulating and non-nodulating soybean plants. Plant Cell Physiol 18: 353–359
14. 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
15. Matsumoto T, M Yatazawa, Y Yamamoto 1977 Effects of exogenous nitrogen-compounds on the concentrations of allantoin and various constituents in organs of soybean plants. Plant Cell Physiol 18: 613–624
16. Mederski HJ, JG Streeter 1977 Continuous automated acetylene reduction assays using intact plants. Plant Physiol 59: 1076–1082
17. Nelson DW, LE Sommers 1973 Determination of total nitrogen in material. Agron J 65: 109–112
18. Pate JS 1962 Root-exudation studies on the exchange of C^{14}-organic substances between the roots and shoot of the nodulated legume. Plant Soil 17: 333–356
19. Pate JS 1973 Uptake, assimilation and transport of nitrogen compounds by plants. Soil Biol Biochem 5: 109–119
20. Pate JS, W Wallace 1964 Movement of assimilated nitrogen from the root system of the field pea (*Pisum arvense* L.). Ann Bot 28: 83–99
21. Reinbothe H, K Mothes 1962 Urea, ureides and guanidines in plants. Annu Rev Plant Physol 13: 129–150
22. Rouch AH, TR Sheih 1962 Diffusion, active transport and metabolism of purines in the yeast *Torulopsis candida*. Biochim Biophys Acta 61: 255–264
23. 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
24. Streeter JG 1979 Allantoin and allantoic acid in tissues and stem exudate from field-grown soybean plants. Plant Physiol 63: 478–480
25. Tajima S, Y Yamamoto 1975 Enzymes of purine catabolism in soybean plants. Plant Cell Physiol 16: 271–282
26. Young EG, CF Conway 1942 On the estimation of allantoin by the Rimini-Schryver reaction. J Biol Chem 142: 839–853