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Effect of Nitrogen Source on Ureides in Soybean[1]

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ABSTRACT

In field-grown soybeans (*Glycine max* L. Merr. cv Harosoy), the percentage of N in the xylem as ureides increased with increasing N_2 fixation. During a 9-week collection period, the ureide content varied from 9.0 to 69.2% of the xylary N. Between 9 and 11 weeks (early pod fill), there was a good correlation ($r = 0.93$) between C_2H_2 reduction and the per cent N in xylem as ureides. The per cent N as ureides, however, does not always indicate the reliance of the plant on symbiotic N_2 fixation. This ureide content also depended on the level of NO_3^- available to the roots. Non-nodulated soybeans given from 0 to 200 kilogram N per hectare produced xylem sap which averaged from 31.8% to 9.0% N, respectively, in the xylem as ureides over the 9-week period.

Feeding of $^{15}N_2$, $^{15}NH_4$, or $^{15}NO_3$ to greenhouse-grown soybeans indicated substantial differences in the initial distribution of N by the xylem stream, but the ultimate distribution of N between plant parts and grain did not vary with available N or percentage of xylary N as ureides. Amino acids, not ureides, were the major source of N in the phloem. The soybeans maintained a similar composition in phloem irrespective of the xylem sap constituents, with N derived from N_2, NH_4, or NO_3 being equally accessible to the phloem stream.

In the xylem of greenhouse-grown soybeans, there are differences between xylary products of N_2 fixation and nitrate reduction (14, 15, 27). The products of N_2 fixation are mostly ureides (allantoin and allantoic acid), whereas amino acids (predominantly asparagine) and NO_3^- result from NO_3^- uptake. Ureides were observed in the xylem sap of field-grown soybeans (26) and were the dominant N compound after 40 d growth, but the relationship between ureides and N_2 fixation or soil N was not determined. During early pod fill, the amount of ureides extracted from shoot tissue of field-grown soybeans is significantly correlated with nitrogenase (C_2H_2) activity (20). Herridge (9, 10) has proposed that the relative ureide content (ureide N/ureide + nitrate N) in soybean tissue can be used to estimate the amount of fixation in the field.

Techniques are now available for collecting phloem sap from soybeans (7, 12) but few studies (11, 13) have determined its composition. Little is known of how the N content of phloem may vary with growth conditions or plant development. We have examined the transport products of N incorporation in xylem and phloem under conditions in which N uptake by soybeans was varied. The consequent partitioning of the N within the plant was determined and compared to differences in the individual constituents of the xylem and phloem to characterize the response of the plant to changes in its N source.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Nodulating and non-nodulating soybean isolines (*Glycine max* L. Merr. cv 'Harosoy') were grown in a greenhouse with supplemental lighting (Multi vapor, 450 μE m^{-2} s^{-1} in a 14:10 h, 27°C:24°C, light:dark regime). Plants were grown two/6-L pot in coarse silica sand, inoculated with Nitragin *Rhizobium japonicum* inoculant, watered twice daily, and given 100 ml of N free nutrient solution (6) three times weekly. The non-nod[3] plants were treated similarly, except the nutrient solution contained either 40 mM KNO_3 or 20 mM $(NH_4)_2SO_4$.

At weekly harvests, petiole phloem sap and xylem sap were collected. Plants were divided into roots, nodules, stems plus petioles, leaflets, apices and flowers, and pods and seeds. Harvests were either four plants or triplicate samples of four plants.

Field-grown plants were sown at Aurora, NY in blocks of four rows, 6 m long × 35 cm apart, at a density of 16 plants/m of row. The center two rows were sampled. The field had previously been sown with corn and, without supplemental N, produced poor growth of non-nod soybeans. We have described estimates of N_2 fixation in immediately adjacent plots (19). P and K were applied to the field at recommended rates before planting. Weeds were controlled by Treflan and hand cultivation. Potassium nitrate was applied to the surface of some of the plots (four blocks per treatment) at weeks 1 and 3 after planting (at total rates of 20 or 100 kg N/ha). Some plots received additional applications of KNO_3 at 20 or 100 kg N/ha at week 8. Plants were harvested weekly from 5 to 13 weeks after planting and at maturity. Ten plants were collected at each harvest, and triplicate samples of 10 plants were taken at the last harvest.

^{15}N Experiments. Greenhouse-grown non-nod plants were given either 200 ml of 99% enriched $(^{15}NH_4)_2SO_4$ (5 mM), or $K^{15}NO_3$ (10 mM) at 54, 64, and 74 DAP. At the same time, nodulated plants were fed ^{15}N-enriched N_2 from which NO had been removed (3). Pots of intact nodulated plants were enclosed in plastic bags which were sealed around the plant stems. The enclosed gas was replaced by a mixture of Ar:O_2 (3:1) which subsequently had ^{15}N-enriched N_2 added to produce a final gas mixture at harvest of approximately 20% (v/v) O_2; 1 to 2% (v/v) CO_2; 50% (v/v) Ar; and 30% (v/v) N_2 (approximately 20 to 30% enriched with ^{15}N). Gas samples were analyzed separately for each pot at the beginning and end of each feeding period on a Micromass 622 mass spectrometer. At either 1.5 or 4 h after application of the ^{15}N sources, the pots were thoroughly leached with nutrient solution and water to remove the label. Samples of four plants were harvested at 1.5 h and 1, 7, and 14 d after application of the ^{15}N. The enrichment with ^{15}N was determined on N_2 gas produced from Kjeldahl digests (21) with a mass spectrometer. Phloem sap and xylem sap samples were collected

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[3] Abbreviations: non-nod, non-nodulating; DAP, days after planting.

from some plants before the leaching of the label, and the acidic, basic, and neutral components were separated by ion exchange resins (1) from the xylem fluid. All fluids were analyzed for sugars, nitrogenous constituents, and ^{15}N enrichment.

Xylem Sap Collections. Xylem exudate was collected for 1 h from cut stems after the surrounding soil had been thoroughly watered. Collections were made from 10 to 15 plants. The pooled sap was frozen and stored for later analysis.

Phloem Sap Collection. Phloem sap was collected from cut petioles or attached pods in 20 mM EDTA, 5 mM KCl, pH 7.0 solutions as described by King and Zeevaart (12) and Fellows *et al.* (7). Collections were made for 2 h after cutting. The excised petioles and attached leaflets were placed in the dark and held at approximately 20°C inside plastic containers to reduce transpiration. Pods remained attached to the plant for the entire collection period. Each sample was the bulked exudate of 6 to 12 petioles or 4 to 6 pods, with triplicate samples taken on several occasions.

Analytical Techniques. Total amino acids were estimated by ninhydrin analysis (17). Total N was determined by Kjeldahl analysis with the salicylic acid modification to include NO_3-N (2). Nitrate was determined by the nitration of salicylic acid in concentrated H_2SO_4 (4). Total sugars in phloem sap were measured using the reaction with phenol in H_2SO_4 (5), and reducing sugars by the copper reduction technique (25). Ureides were measured using differential hydrolysis followed by colorimetric estimation of the glyoxylic acid produced (28). Because the sum of the NO_3, ureides, and amino acids accounted for over 90% of the total N determined by Kjeldahl, this sum was used for calculating the percentage composition of the sap samples.

Nitrogenase (C_2H_2) was assayed on triplicate samples of two roots from which the soil was lightly shaken. The roots were placed in l-L jars, and C_2H_2 added to 10% (v/v). Gas samples were taken 10 and 40 min later, and C_2H_4 analyzed by GC.

RESULTS AND DISCUSSION

Greenhouse Plants. Samples of xylem exudate taken weekly from plants between 40 and 75 DAP averaged 87%, 18%, and 28% of N as ureides for the plants grown with N_2, NO_3^-, and NH_4^+, respectively. Amino acids averaged 11%, 40%, and 66% for the same samples with NO_3^- comprising 2%, 4%, and 5% of the xylary N. Ureides were lowest and amino acids highest during the middle of the collection period. This agrees with other reports that the form of N in transport compounds of greenhouse-grown soybeans depends on the form in which N is supplied (15) and that most of the ureides are produced in the nodules (8, 24).

The high amino acid content of xylem sap taken from NO_3^--grown plants supports reports that some NO_3^- reduction takes place in soybean roots (15, 22). The xylem sap showed an enrichment of ^{15}N in the reduced fraction 1.5 h after beginning feeding with $^{15}NO_3^-$ (Table I). This enrichment was higher than in the phloem sap and was not therefore recycled N.

The N distribution into plant parts was similar among treatments. From nine Kjeldahl determinations between 41 and 64 DAP, leaves averaged 53.8 ± 0.5% (SE), 50.5 ± 2.2%, and 53.7 ± 0.7% of the plant N for N_2, NO_3^-, and NH_4^+ grown plants, respectively. The stems averaged 12.0 ± 0.9%, 9.3 ± 0.7%, and 11.8 ± 0.8% of the plant N for the same period and treatments. This means that the distribution of N above and below ground was similar for the treatments. However, the N_2-grown plants had 39% of the below ground N in the nodules. The per cent N in the roots of both N_2 (1.56 ± 0.05% [SE]) and NO_3^- (1.53 ± 0.05%) grown plants did not differ though the roots were smaller on the N_2-grown plants. Root growth by plants relying on N_2 fixation may be restricted (as suggested previously for *Lupinus albus* [18]), because of their increased requirement for photosynthate (23).

Table I. *^{15}N Enrichment of the Transport Fluids and Plant Parts after Feeding ^{15}N-Labeled Substrates to the Roots of 54-Day-Old Greenhouse-Grown Soybeans*

Each sample consisted of the bulked material from four plants. (Enrichment values are all corrected to 100% enrichment in the substrate). The tissue samples were extracted with 50 mM phosphate buffer. Feeding was begun 1.5 h prior to collection and continued during the 1-h collection.

Collection Location	Enrichment of ^{15}N from Following Sources		
	$^{15}N_2$	$^{15}NO_3^-$	$^{15}NH_4^+$
	%		
Xylem sap (total)	27.0	15.1	15.0
Xylem sap (acidics + neutrals)[a]	31.9	28.9	10.8
Xylem sap (basics)[b]	8.4	11.2	16.0
Petiole phloem sap	2.5	3.0	3.7
Soluble stem N	0.6	1.5	0.9
Soluble leaf N	0.4	2.0	0.6

[a] Primarily ureides.
[b] Primarily amino acids.

Table II. *Distribution of ^{15}N among Shoot Parts of Glasshouse-Grown Soybeans Fed Different ^{15}N Sources to the Roots for 1.5 Hours Immediately Prior to Harvest*

Values are the percentage of the total ^{15}N reaching the shoot ± SE of four plants at each harvest. At 64 d, the plants were bulked prior to analysis and no SE was calculated.

^{15}N Source	Plant Age	Stem	Leaflets	Pods	Axilliaries
	d				
$^{15}N_2$	54	40.6 ± 1.2	58.6 ± 7.0	0.39 ± 0.09	0.56 ± 0.28
	64	53.8	44.6	0.96	0.61
$^{15}NO_3^-$	54	15.5 ± 2.5	82.9 ± 3.4	0.92 ± 0.25	0.73 ± 0.14
	64	15.3	84.2	0.31	0.18
$^{15}NH_4^+$	54	41.3 ± 5.1	56.8 ± 1.9	1.4 ± 0.60	0.55 ± 0.18
	64	42.6	56.7	0.38	0.25

The initial distribution of ^{15}N was very different in plants grown on NO_3^- compared to those grown on either N_2 or NH_4^+ (Table II). A considerable accumulation in the stem occurred only on the latter two forms of N. The similarity of relative enrichments of the phloem sap, however, indicated a similar proportion of the incorporated N was transferred from the xylem to phloem in all cases in spite of differences in the N distribution and form of the supplied N (Table I).

In spite of the different initial distributions of N shown by the short term ^{15}N feeding experiment (Table II), a similar final partitioning was achieved for the three N sources when the ^{15}N was traced for 2 weeks after feeding (Fig. 1). For example, leaves and roots of NO_3^--grown plants lost considerable ^{15}N with time whereas stems increased in ^{15}N content. This process was reversed for N_2-grown plants.

A number of factors were examined to determine how these differences in redistribution could have arisen. Apparently, composition of the phloem sap was independent of the source of N (Table III) with the ureides comprising a substantial, but not dominant, proportion in all cases. Three duplicated collections between 54 and 74 d of age for leaflets 2 to 6 contained 29 ± 10% (SE), 32 ± 9%, and 32 ± 8% of the phloem N in ureides for NH_4^+, NO_3^-, and N_2-grown plants, respectively. This occurred despite up to 5-fold differences in percentage of N as ureides in the xylem over this period.

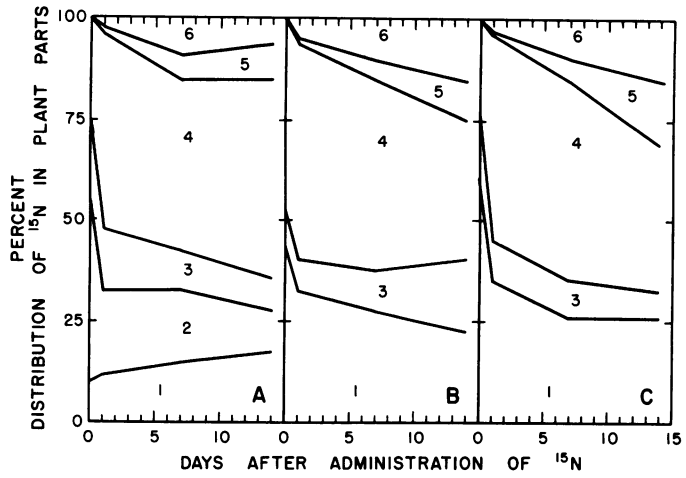

FIG. 1. Distribution of ^{15}N in plant parts 1, 7, and 14 d after feeding ^{15}N as N_2 (A), NO_3^- (B), or NH_4^+ (C). The parts 1 (root), 2 (nodules), 3 (stems), 4 (leaflets), 5 (axils), and 6 (pods and seeds) were analyzed as described in the text.

Table III. *Phloem Composition of Leaflets 4 to 8 on 78-Day-Old Plants*

Collections were made in EDTA solutions for 2 h after cutting the petiole. Each sample was four leaves from two plants, measured in triplicate. Each result is the mean ± SE of four samples.

Phloem Constituent	N Source for Growth		
	NH_4^+	NO_3^-	N_2
Sugars, μg leaf^{-1} min^{-1}	1.60 ± 0.28	0.92 ± 0.16	0.93 ± 0.37
Amino acids, nmol leaf^{-1} min^{-1}	1.02 ± 0.34	1.17 ± 0.35	1.16 ± 0.37
Ureides, nmol leaf^{-1} min^{-1}	0.38 ± 0.14	0.26 ± 0.04	0.27 ± 0.06

Field experiments. Flowering began 7 weeks after planting and by 13 weeks the plants averaged 65% podfill. The final harvest data for the plants are given in Table IV (roots and fallen leaflets and petioles not included). Figure 2 shows the nitrogenase (C_2H_2) activity from 5 to 13 weeks after planting. For clarity, results from the middle level of N availability (20 kg N ha^{-1}) are omitted; they lie between the 0 and 100 kg N ha^{-1} values. The decreased nitrogenase activity at week 7 could be a result of dry weather between weeks 4 and 7 (19).

Figure 3A shows the per cent N as ureides in xylem of nodulated soybeans. Again, data for 20 kg N ha^{-1} are omitted. The average values of the per cent N as ureides in xylem for the entire collection period are given in Table IV. There is a close similarity during growth in the patterns of nitrogenase activity (Fig. 2) and the per cent N as ureides in xylem (Fig. 3A). When nitrogenase activity was maximum (weeks 9–11), the regression of acetylene reduction activity on per cent N as ureides gave a strong correlation ($r = 0.93$).

The relationship of xylem ureides to N_2 fixation observed in greenhouse-grown soybeans (14, 15) is therefore confirmed with field-grown soybeans. The results are similar to those obtained by analysis of ureides in shoot tissue; during early podfill, there is a strong correlation between the concentration of ureides in shoot and the acetylene reduction activity of roots (20).

Figure 3B shows the ureide contribution to xylem N in non-nod plants. It is apparent that ureides in xylem are not necessarily linked to N_2 fixation. The lack of adequate levels of N in the soil (resulting in up to an 87% reduction in seed yield; Table IV) also leads to an increase in ureide percentage in the non-nod plants (Fig. 3B). Non-nod plants under N stress often exceeded some

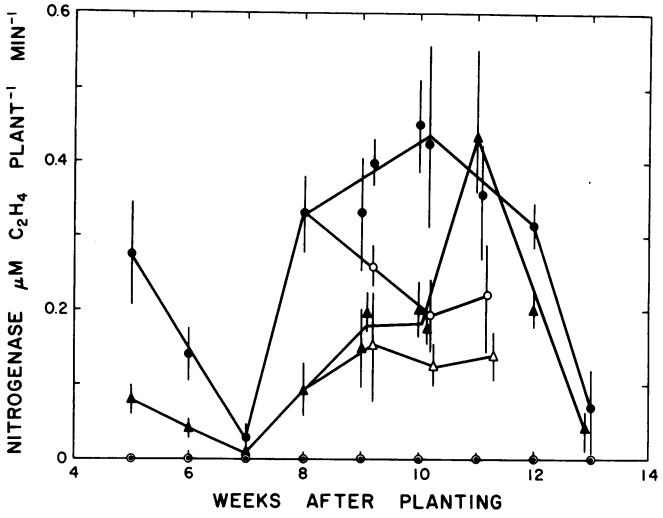

FIG. 2. Nitrogenase activity of field grown non-nodulated (◉) or nodulated soybean plants either untreated (●) or fed 50 kg N/ha as KNO_3 at weeks 1 and 3 (▲) and/or 100 kg N/ha at week 8 (○,△). Bars indicate SE.

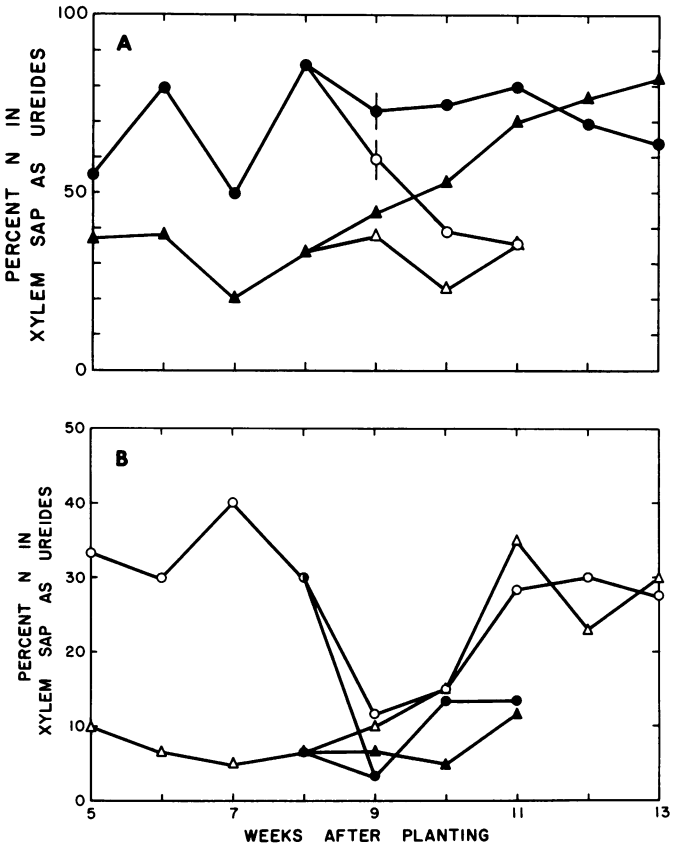

FIG. 3. A, Percentage of xylem N as ureides during the life cycle of field-grown nodulated soybeans. Plants were either untreated (●) or fertilized with 50 kg N/ha as KNO_3 at weeks 1 and 3 (▲), and/or 100 kg N/ha at week 8 (○,△). Bars indicate SE. B, Percentage of xylem N as ureides during the life cycle of field grown non-nodulating soybeans. Plants were either untreated (○) or fertilized with 50 kg N/ha as KNO_3 at weeks 1 and 3 (△), and/or 100 kg N/ha at week 8 (▲,●).

of the nodulated treatments for percentage of ureides in the xylem N. Table IV shows that, averaged over the entire collection period, the non-nod plants without added N equaled (31.8%) the xylary ureide percentage of the nodulated plants given the

Table IV. *Harvest Data for all Treatments on Field-Grown Soybeans*

Nod	N Added		N in Seed	Seed N	Pod N	Stem N	N from Fixation	N Harvest Index	N as Ureides in Xylem
	Weeks 1 & 3	Week 8							
	kg/ha		%		*g/plant*		%		%
+[a]	0	0	7.32 ± 0.08	0.82 ± 0.16[b]	0.038 ± 0.006[b]	0.026 ± 0.004[b]	82.9[c]	92.7[d]	69.2[e]
+	0	100	7.36	1.007	0.048	0.041	53.7	91.9	54.0
−	0	0	4.61 ± 0.09	0.135 ± 0.017	0.005 ± 0.001	0.011 ± 0.003	0	89.4	31.8
−	0	100	6.41	0.478	0.020	0.009	0	94.3	24.4
+	20	0	7.16 ± 0.08	0.78 ± 0.06	0.034 ± 0.003	0.031 ± 0.002	66.5	92.3	62.1
+	20	20	5.54	0.55	0.039	0.051	56.0	86.0	54.7
−	20	20	5.17	0.250	0.015	0.016	0	89.0	29.6
+	100	0	7.30 ± 0.10	0.77 ± 0.10	0.035 ± 0.005	0.045 ± 0.006	59.4	90.3	45.5
+	100	100	7.30	0.89	0.048	0.049	44.6	90.2	31.8
−	100	0	5.25 ± 0.07	0.31 ± 0.01	0.016 ± 0.001	0.200 ± 0.002	0	89.5	15.8
−	100	100	6.12	0.50	0.026	0.021	0	91.4	9.0

[a] +, nodulated plants; −, non-nodulated plants.

[b] Values are means of three replicates ± SE of samples containing 10 plants. Values without SE had the 30 plants bulked before analysis. Standard errors varied between 3 and 23% of the mean N value and averaged 13% of the mean N value.

[c] (Total N nod plants − total N non-nod plant)/(total N non plant) × 100.

[d] Harvest index is the total plant N that is in the seed, but does not include N loss in fallen petioles and leaflets or in roots and nodules.

[e] Per cent ureides is the average percentage for the entire period of collection, weighing each week's collection equally.

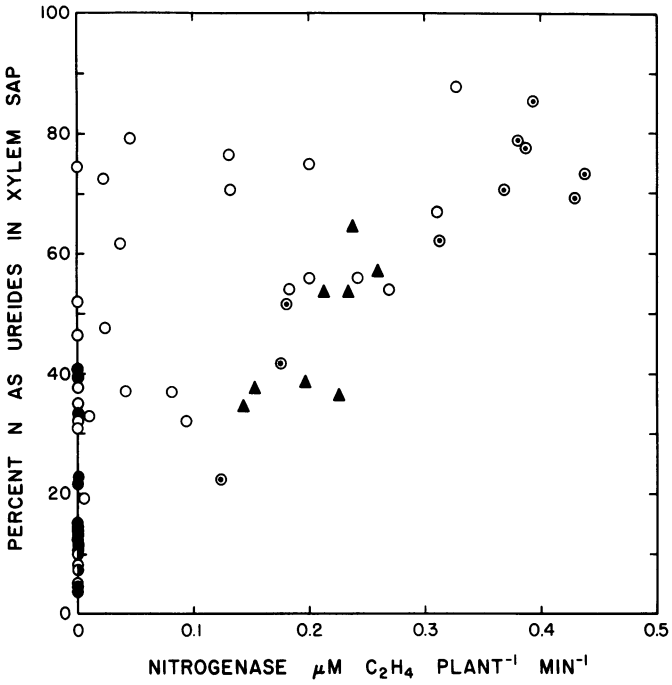

FIG. 4. Relationship between xylary ureide content and acetylene reduction in field-grown soybean plants. Solid symbols represent all samples taken between weeks 9 and 11 after planting. Open circles (O) represent all other times for the same treatments from weeks 5 to 13. (●), Non-nodulated plants given 0, 20, 40, 100, or 200 kg N/ha as KNO_3 at the same times as for the nodulated plants. (◉), Nodulated plants given 0, 10, or 50 kg N/ha at weeks 1 and 3. (▲), nodulated plants given an additional 20 or 100 kg N/ha at week 8.

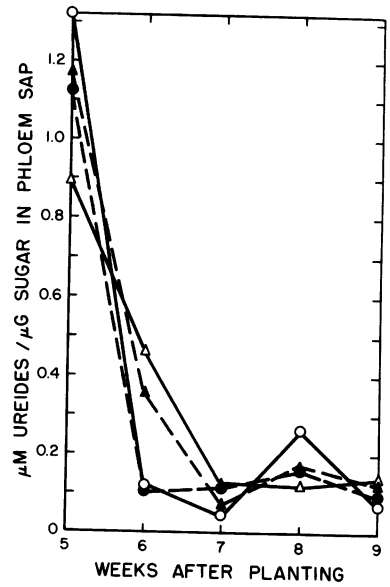

FIG. 5. Ureide content of phloem sap collected from petioles 4 and 5 of field-grown soybean plants. Nodulated (O) and non-nodulated (Δ) plants were used, either untreated or fed 50 kg N/ha as KNO_3 at weeks 1 and 3 (●,▲). Phloem sap was the exudate from cut petioles into a 20 mM (pH 7.0) EDTA solution. Collection was in the dark for 2 h after cutting.

highest level of added NO_3^-. This was in spite of substantial acetylene reduction activity in the latter (Fig. 2). Thus, the linkage between percentage ureides in the xylem sap and acetylene reduction was not independent of other factors affecting the plant (Fig. 4). We have shown (20) that source-sink manipulations do not always change nitrogenase (C_2H_2) activity and ureide content of shoot tissue in the same way.

The proportion of xylary N as ureides depended on three factors: (a) the availability of soil N; (b) the production of ureides by the roots; (c) the production of ureides by the nodules. The calculated export of ureides of non-nod roots appeared to be a constant factor among treatments. Between weeks 5 and 9, it averaged 1.50, 1.61, and 1.21 mg ureide N plant^{-1} d^{-1} at levels of applied NO_3^- of 0, 20, and 100 kg N/ha. (The values are determined as the appropriate N increments multiplied by the corresponding percentage ureides in the xylem sap [Fig. 3].) The export rate in non-nods was, therefore, low compared to nodulated plants (which reached up to 10.3 mg ureide N plant^{-1} d^{-1} for the most actively fixing plants). Nevertheless, it was sufficient to produce a high ureide percentage in the sap of N-deficient plants.

In spite of this great range in estimated reliance on fixation and percentage N as ureides in the xylem for nodulated plants, the per cent N in the seed at harvest showed no significant

difference between NO_3^- treatments, except when small, late additions of NO_3^- inhibited fixation without supplying an adequate alternate source of N. In this situation the per cent N in the seed was reduced to a level similar to that of non-nod plants, which produced similar yields (Table IV).

Total yield was also unaffected by the proportion of N reaching the shoots as ureides. For nodulated plants, there was no significant chage in yield for plants receiving from 31.8% to 69.2% of their N as ureides (Table IV). Yield and per cent N in the seed seemed to be a consequence of the total N incorporated by the plant, but not its source.

The distribution of N between the above ground parts was also constant, in agreement with the N harvest index (Table IV) for nodulating (90 ± 1.1 (SE)) against non-nod (90.7 ± 1.1) plants between high (91.3 ± 0.8) and low/medium (89.9 ± 1.3) fertilizer treatments. This was in spite of average differences in the percentage of ureides in the xylem of 2.4-fold and extreme differences of up to 8-fold. This shows that the form in which N reached the shoot did not affect its subsequent redistribution to the seed.

The partitioning of N during growth agreed with the results for greenhouse-grown plants. Table IV shows that this partitioning was independent of the reliance of the plant on fixation and also independent of the amount of N available to the plant. For example, the mean percentage of the plant N in the stem between weeks 5 and 13 was 20.0 ± 2.3 (SE), 21.4 ± 2.5, 21.9 ± 1.6, and 25.8 ± 2.3 for non-nod and nodulated plants without supplemental N followed by the values for plants with 100 kgN/ha added (Table IV).

Figure 5 gives values for phloem ureides for the same four treatments given in Figure 2. The wide range of xylary ureides encompassed by these treatments (Table IV) apparently had no effect on the phloem ureide level. This was true not only for petiole phloem sap but also for that collected from the fruit tip. Analyses of petiole and fruit tip phloem sap collected at node 7 between 9 and 11 weeks gave 0.63 ± 0.10 (6), 0.55 ± 0.10 (6), (fruit tip, nod, and non-nod), and 0.31 ± 0.05 (8), (petiole, nod, and non-nod) nmol of ureides per μg sugars in the phloem (values are means ± SE with number of replicates in parentheses). The amino content of the phloem sap was more variable; the averages of six to eight samples taken between weeks 5 and 9 from nodes 4 and 5 were 0.84 ± 0.15 (SE), 0.81 ± 0.12, and 1.59 ± 0.40 nmol amino acids/μg sugar for 0, 20, and 100 kg/ha treated nodulated plants. For the corresponding non-nodulated plants, values was 0.81 ± 0.07, 0.70 ± 0.18, and 1.06 ± 0.31.

That the phloem composition is kept stable is, therefore, indicated in both greenhouse- and field-grown plants. The possibility of stem loading of N compounds (found previously in *L. albus* [18]) is suggested for ureides independent of the xylary ureide level. Evidence supporting this is presented elsewhere (13).

Our results confirm the relationship between ureides in xylem and N_2 fixation for greenhouse-grown soybeans (14, 15). However, the situation in the field was more complicated. A single measure of percentage N as ureides did not always give a good estimate of fixation. It may therefore be premature to use ureide content as a measure of the amount of N fixed by crops (9, 10).

Under all conditions studied, the xylem contained a substantial quantity of ureides recently produced from newly incorporated N (Table II) by non-nod roots. This suggests a situation similar to *L. albus* (16) in which different parts of the plant used different xylary N sources for growth. The reduced N in the xylem fluid of plants growing on NO_3^- may represent a specific source of N for stem growth as it was initially more strongly removed by the stem.

Why the nodules should produce ureides as a major source of xylary N while the root produces only small amounts is not clear. Other studies (27) have indicated that xylary composition of reduced N does not always depend on the source of N (either N_2 or NO_3^-) in legumes. To compensate for these differences in the xylem sap, major metabolic changes must take place in the soybean shoot. To clarify these metabolic alterations the inputs, outputs, and accumulations of ureides and other metabolites must be measured throughout the life cycle for individual portions of the soybean plant (13).

Ultimately, the plant can compensate for changes in N source, with the result that per cent N in the seed and yield depend only on the total amount of N incorporated by the entire plant. Indeed, distribution of N within the plant, and N harvest index depend on neither the form nor the amount of supplied N. The composition of the seeds is maintained by the phloem sap which is the major source of seed N (18) and which had a constant composition under all the conditions we tested. Such a phenomenon may be common in legumes. How the factors involved in secondary distribution are modified to compensate for the changes in xylary composition are still unknown.

LITERATURE CITED

1. ATKINS CA, DT CANVIN 1971 Photosynthesis and CO_2 evolution by leaf discs: Gas exchange, extraction, and ion-exchange fractionation of ^{14}C-labelled photosynthetic products. Can J Bot 49: 1225–1234
2. BREMNER JM 1965 Total Nitrogen. *In* CA Black, ed, Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison WI, pp 1149–1176
3. BURRIS RH 1974 Methology. *In* A Quispel, ed, The Biology of Nitrogen Fixation. North Holland Publishing Company, Amsterdam
4. CATALDO DA, M HAROON, LE SCHRADER, VL YOUNGS 1975 Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. Commun Soil Sci Plant Analysis 6: 71–80
5. DUBOIS M, KA GILLES, JK HAMILTON, PA REBERS, F SMITH 1956 Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350–356
6. EVANS HJ, B KOCH, R KLUCAS 1972 Preparation of nitrogenase from nodules and separation into components. Methods Enzymol 24B: 470–476
7. FELLOWS RJ, DB EGLI, JE LEGGETT 1978 A pod leakage technique for phloem translocation studies in soybean (*Glycine max*[L] Merr.). Plant Physiol 62: 812–814
8. FUJIHARA S, M YAMAGUCHI 1978 Effects of allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine) on metabolism of allantoin in soybean plants. Plant Physiol 62: 134–138
9. HERRIDGE DF 1982 Relative abundance of ureides and nitrate in plant tissues of soybean as a quantitative assay of nitrogen fixation. Plant Physiol 70: 1–6
10. HERRIDGE DF 1982 Use of the ureide technique to describe the nitrogen economy of field-grown soybeans. Plant Physiol 70: 7–11
11. HOUSLEY TL, DM PETERSON, LE SHRADER 1977 Long distance translocation of sucrose, serine, leucine, lysine and CO_2 assimilates. I. Soybean. Plant Physiol 59: 217–220
12. KING RW, JAD ZEEVAART 1974 Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol 53: 96–103
13. LAYZELL DB, TA LARUE 1982 Modeling C and N transport to developing soybean fruits. Plant Physiol 70: 1290–1298
14. MATSUMOTO T, Y YAMAMOTO, M YATAZAWA 1976 Role of root nodules in the nitrogen nutrition of soybeans. II. Fluctuations in the allantoin concentration of the bleeding sap. J Sci Soil Manure 47: 463–469
15. MCCLURE PR, DW ISRAEL 1979 Transport of nitrogen in the xylem of soybean plants. Plant Physiol 64: 411–416
16. MCNEIL DL, CA ATKINS, JS PATE 1979 Uptake and utilization of xylem-borne amino compounds by shoot organs of a legume. Plant Physiol 63: 1076–1081
17. MOORE S, WH STEIN 1948 Photometric ninhydrin method for use in the chromatography of amino acids. J Biol Chem 176: 367–388
18. PATE JS, DB LAYZELL, DL MCNEIL 1979 Modelling the transport and utilization of carbon and nitrogen in a nodulated legume. Plant Physiol 63: 730–737
19. PATTERSON TG, TA LARUE 1983 Nitrogen fixation (C_2H_2) by soybeans: cultivar and seasonal effects, and a comparison of estimates. Crop Sci 23: 488–492
20. PATTERSON TG, TA LARUE 1983 Dinitrogen fixation and ureide content of soybeans: ureides as an index of fixation. Crop Sci 23: 825–831
21. RITTENBERG D, AS KESTON, F ROSEBURY, R SCHOENHEIMER 1939 The determination of nitrogen isotopes in organic compounds. J Biol Chem 127: 291–299
22. RUFTY TW JR, RJ VOLK, PR MCCLURE, DW ISRAEL, CD RAPER JR 1982 Relative content of NO_3^- and reduced N in xylem exudate as an indicator of root reduction of concurrently absorbed $^{15}NO_3^-$. Plant Physiol 69: 166–170
23. RUSSELL WJ, DR JOHNSON 1975 Carbon-14 assimilate translocation in nodu-

lated and non-nodulated soybeans. Crop Science 15: 159–161
24. SCHUBERT KV 1981 Enzymes of purine biosynthesis and catabolism in *Glycine max*. Plant Physiol 68: 1115–1122
25. SOMOGYI M 1952 Notes on sugar determination. J Biol Chem 195: 19–23
26. STREETER JG 1979 Allantoin and allantoic acid in tissues and stem exudate from field-grown soybean plants. Plant Physiol 63: 478–480
27. THOMAS RJ, LE SCHRADER 1981 Ureide metabolism in higher plants. Phytochemistry 20: 361–371
28. VOGELS GD, C VAN DER DRIFT 1970 Differential analyses of glyoxylate derivatives. Anal Biochem 33: 143–157