Amer. J. Bot. 77(3): 316–322. 1990.

FREE AMINO ACID POOLS, $^{15}N_2$ FIXATION AND FIXED N_2 ASSIMILATION IN LEUCAENA LEUCOCEPHALA VAR. K-8[1]

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ABSTRACT

Various organs of *Leucaena leucocephala* (Lam.) de Wit were analyzed for their levels of total nitrogen and free amino acids as well as for changes in free amino acid pools from the time of germination through nodulation. Also an assessment was made of the sink of fixed N_2 (transport product) in the nodules using ^{15}N methodology. *L. leucocephala* organs showed total nitrogen levels similar to those of other legumes. Asparagine was the most prevalent amino acid in the nodules and roots followed by glutamate and mimosine. Asparagine was the second most common amino acid in the leaves and stems, with mimosine being the most abundant. Strong correlations were found between the total plant levels of aspartate and glutamate, asparagine and NH_4^+, acetylene reduction and glutamate, and asparagine and plant age. Asparagine amino- and amide-N accounted for over 75% of the fixed $^{15}N_2$ in nodules. It was concluded that *L. leucocephala* is an asparagine transporter of fixed N_2 in the nodule.

LEUCAENA LEUCOCEPHALA (Lam.) de Wit (= *Leucaena glauca* Benth.) is rapidly becoming an economically and ecologically important legume in the tropics because of its multiple uses (National Academy of Sciences, 1980). Of the ten recognized species in the *Leucaena* genus, only *L. leucocephala* has been exploited to date (Brewbaker, 1983). *Leucaena* currently is used for forage, firewood, timber, pulp, revegetation, soil improvement, and the control of soil erosion. The only drawbacks in extensive use of this legume appear to be its ability to become a weed in some areas and the presence of mimosine at levels as high as 6.4% of the foliage dry matter (Matsumoto, Smith, and Sherman, 1951). Mimosine has been reported to be toxic to ruminants when consumed in excessive amounts (National Academy of Sciences, 1980), but a later report of the National Research Council (1984) indicated that ruminants in Indonesia, India, and Hawaii thrived on diets consisting of 100% *Leucaena* plus a salt supplement. The leaflets of this plant contain 25–30% protein and are approximately 70% digestible (National Research Council, 1984).

The ability of *Leucaena* to fix N_2 has been well documented; annual rates of fixation as high as 100 to 200 kg N/ha have been reported (Hogberg and Kvarnstrom, 1982; National Research Council, 1984; van Kessel et al., 1983). Most estimates of total annual N_2 fixation are based on the measurement of acetylene reduction activity, assuming that 3 moles of acetylene reduced are equal to 1 mole of N_2 fixed. Van Kessel et al. (1983), however, showed that the conversion factor for *Leucaena leucocephala* was significantly less than 3.0. No reports have appeared in the literature regarding the free amino acid pools (other than mimosine) in the organs or tissues of *Leucaena* or in what compound fixed N_2 is transported from the nodule. The objectives of this study were 1) to determine the nitrogen content of and the free amino acid pools in various organs of *L. leucocephala,* 2) to measure any changes in the concentrations of free amino acids in the plants from the time of germination through nodulation, and 3) to identify the N compound exported from nodules.

MATERIALS AND METHODS—*Plant material*—Seeds of *Leucaena leucocephala* (Lam.) de Wit variety K-8 were obtained from NifTAL (Paia, HI) and a *Leucaena*-specific *Rhizobium* inoculent was obtained from Nitragin (Milwaukee, WI). Seeds were nicked with a single-edged razor blade and were allowed to imbibe distilled water for 1 hr prior to sowing. Imbibed

[1] Received for publication 30 January 1989; revision accepted 14 September 1989.

This research was funded by a College Instructor fellowship to JDD from the American Society of Biological Chemists, Summer 1986.

The authors thank Dr. R. H. Burris for allowing us to use the mass spectrometer facility at the University of Wisconsin–Madison. A small portion of this research was conducted in Dr. Burris' laboratory.

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seeds were planted in 15-cm diameter pots containing vermiculite or vermiculite : perlite (1:1, v/v). All seeds were germinated and the plants grown in the greenhouse. Plants were watered daily and beginning 2 weeks after germination, plants were fertilized twice weekly with an aqueous fertilizer described by Summerfield, Huxley, and Minchin (1977), modified to exclude nitrogen. One-year-old plants were used for total organ N, 1- to 7-week old plants were used to assess N content from germination through nodulation. Plants 4- to 5-months old were used for ^{15}N experimentation.

Extraction of plant tissues and the determination of free amino acid pools—Leaves, stems, roots, and nodules from individual plants were placed in separate screw-capped test tubes containing 10 ml of 0.5% (v/v) toluene in water (Winter and Dekker, 1984). The tubes were heated for 15 to 20 min on a steam bath to inactivate enzymes; they were then cooled and the tissues allowed to extract at room temperature for 6 to 8 hr. At the end of the extraction period, 2-aminobutyric acid (1.0 μmol/g fresh wt) was added as an internal standard and the contents of the tubes were then filtered. The filtrates were concentrated to dryness in vacuo at 45 C and the residues redissolved in 1 ml of water. A sample of each of these extracts was diluted with Na citrate-HCl buffer (pH 2.2) and analyzed using a Beckman Model 120B amino acid analyzer modified for three-buffer operation. Na citrate-HCl buffers were used with pH values of 3.25, 4.25, and 6.40.

Acetylene reduction assay for N_2 fixation—Nodules freshly harvested from each plant were placed in separate 20-ml Wheaton vaccine bottles fitted with rubber vaccine stoppers. Acetylene was then injected into each bottle to yield a 10% (v/v) acetylene atmosphere. After 1 hr, a sample of the gas phase was analyzed with a Gow Mac series 750 GC containing a column of Porapak N (1.8 m × 3 mm diam) and a flame ionization detector.

Fixation of $^{15}N_2$—Nodules freshly harvested from each plant were placed in separate 20-ml Wheaton vaccine bottles fitted with rubber vaccine stoppers. The bottles were connected to a manifold with hypodermic needles and the gas phase of each was evacuated and flushed with argon three times. After the final evacuation, the bottles were filled with an atmosphere containing 20% (v/v) $^{15}N_2$ (99 atom % ^{15}N), 20% (v/v) O_2 and 60% (v/v) Ar. $^{15}N_2$ was generated by the method outlined by Burris (1976). Control bottles were filled with an atmosphere of air. Experiments typically lasted for 1–1.5 hr.

Determination of N content and ^{15}N enrichment—Plant organs (nodules, roots, stems, and leaves) and soluble fractions therefrom (except the free NH_4^+ fractions) were subjected to Kjeldahl digestion and distillation (Burris and Wilson, 1957). A small sample of each distillate and of each free NH_4^+ fraction was used for determining ammonia-N by Nessler's reaction (Burris and Wilson, 1957) and the rest was concentrated, converted to N_2 by hypobromite oxidation and then analyzed for ^{15}N with a MAT 250 isotope ratio mass spectrometer.

Analysis of ureide and total amino acid N—Thirty nodules were harvested from each of eight 4-month-old plants. Six groups of nodules were exposed to $^{15}N_2$ for 1 hr as described above, while two control groups were exposed to air. After this treatment, each group of nodules was removed from the vaccine bottle, weighed, and ground in a mortar with 5 ml of 70% (v/v) ethanol. The resulting homogenates were centrifuged 15 min at 10,000 × g, the pellets were resuspended in 5 ml of 70% ethanol, and the mixtures again centrifuged. This pellet was resuspended in 5 ml of water and the suspension centrifuged a third time. The supernatant solution and washings from each sample were combined, diluted to 25 ml with distilled water, and applied to a column of Dowex-50X8 (H^+ form, 1 × 15 cm) which was then rinsed with water. The rinse solution (40 ml) was collected and constituted the ureide fraction. Amino acids were then eluted from the column with 100 ml of 1 N NH_4OH. The eluate (amino acid fraction) was evaporated to dryness at 45 C in a fume hood and the resulting residue subsequently redissolved in a small volume of 0.02 N HCl.

Ureides were determined using 5 ml of the appropriate fraction by the procedure of Young and Conway (1942), modified such that the final volume was 15 rather than 25 ml. The remaining 35 ml of the ureide fractions, the amino acid fractions, and the pellets were subjected to Kjeldahl digestion and distillation for the determination of the N content and the level of ^{15}N enrichment (see above).

Separation of NH_4^+, amino-N and amide-N from acidic amino acids and their amides—A portion of an extract of excised nodules exposed to $^{15}N_2$ was passed through a column of Dowex-1X8 (acetate form, 0.7 × 5.0 cm), which was then rinsed with 3 column volumes of

TABLE 1. *Levels of N in organs of* L. leucocephala *var. K-8*

Organ[a]	No. of replicates[b]	N content	
		mg/organ	% (w/dry wt)
		(mean ± SE)	
Nodules	3	0.63 ± 0.03	7.2 ± 0.4
Roots	3	nd[c]	1.8 ± 0.1
Stems	9	nd	1.5 ± 0.1
Leaves	10	31.3 ± 5.2	3.3 ± 0.2
Flowers	2	9.3 ± 0.6	4.1 ± 0.0
Seeds	5	2.7 ± 0.3	4.2 ± 0.1

[a] All tissues except seeds were from 1-year-old plants.
[b] Replicates = no. plants for nodules, roots, stems; no. organs for leaves, flowers, seeds.
[c] nd = not determined.

deionized water to remove neutral and basic amino acids. The acidic amino acids were eluted from the column with 0.5 N acetic acid. This fractionation procedure was carried out in 3 separate batches for each sample; thereafter, the corresponding fractions were combined and concentrated to dryness in vacuo. The residues containing the acidic amino acids were redissolved in Na citrate-HCl buffer (pH 2.2) and fractionated on the amino acid analyzer. Those fractions containing aspartic acid and glutamic acid were collected directly from the column, without ninhydrin reaction, at their appropriate retention times.

The solution containing the neutral and basic amino acids was concentrated to dryness in vacuo and the residue redissolved in 1 ml of water. The free ammonia present in this solution was collected by microdiffusion following the addition of 1 drop of a saturated solution of K_2CO_3 (Burris, 1972). After diffusion of the free ammonia was complete, the solution was adjusted to pH 6.5 with $HClO_3$, the $KClO_3$ that precipitated was removed and discarded, and the resulting neutralized, K-free solution was concentrated to dryness in vacuo. The residue was redissolved in 0.1 M Na acetate buffer (pH 4.9) and this solution was treated for 30 min with 0.5 unit of glutaminase (EC 3.5.1.2; 5 U/ml, Sigma Chem. Co.). The ammonia released from glutamine was collected by microdiffusion. The remaining solution was treated and concentrated as before, the residue was dissolved in 0.1 M Na acetate buffer (pH 8.5), and the asparagine still present was hydrolyzed by incubating for 1 hr with 0.5 unit of asparaginase (EC 3.5.1.1; 5 U/ml, Sigma Chem. Co.). Thereafter, the final solution was fractionated on a column of Dowex-1X8, and the ammonia (from asparagine) as well as aspartic acid and glutamic acid (from asparagine and glutamine) were recovered as described above.

RESULTS—The total nitrogen content found in organs of *L. leucocephala* (mg N and weight % N) is given in Table 1. Although the nodules of 1-year-old plants averaged 7.2% N, samples of nodules at other ages contained from 3.6% N (2 months old) to 8.7% N (5 months old). The percentage of N in roots and stems was consistently lower than in other tissues over the course of the experiment. The value for leaves of 1-year-old plants is representative of leaves in all samples 4 months or older; leaves from younger plants contained from 2.2 to 3.0% N.

Table 2 shows the levels of free amino acids

TABLE 2. *Free amino acid contents in organs from 4.5-month-old* L. leucocephala *trees grown on N-free nutrient solution*

Amino acid	μmol/g fresh wt[a]			
	Leaves	Stems	Roots	Nodules
Asp	1.3 ± 0.02	0.4 ± 0.02	0.3 ± 0.03	1.3 ± 0.1
Asn[b]	8.5 ± 0.3	6.2 ± 1.4	5.9 ± 1.9	27.3 ± 2.3
Glu	0.7 ± 0.1	0.3 ± 0.03	0.4 ± 0.04	4.8 ± 0.6
Gly	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.00	0.5 ± 0.1
Ala	1.2 ± 0.1	0.6 ± 0.03	0.5 ± 0.02	2.7 ± 0.2
Mim[c]	13.1 ± 1.9	9.0 ± 1.4	5.0 ± 1.1	3.8 ± 0.7
GABA[d]	3.1 ± 0.4	1.5 ± 0.1	0.9 ± 0.1	3.2 ± 0.4
NH_4^+	3.2 ± 0.4	0.4 ± 0.05	0.9 ± 0.1	3.4 ± 0.5
Arg	1.9 ± 0.01	0.3 ± 0.07	0.2 ± 0.1	nd[e]
Total	34.3	19.2	14.6	48.5

Pro, Val, Leu, Tyr, His, and Lys present in trace amounts (<0.5 μmol/g fresh wt). Met and Phe—nd.
[a] Mean ± SE of 3 samples.
[b] Includes Gln, Ser, and Thr (see Table 3).
[c] Mimosine.
[d] γ-Aminobutyric acid.
[e] nd = not determined (see text).

TABLE 3. *Effect of acid- and enzymatic hydrolysis of amides on the levels of acidic amino acids/amides in nodule extracts from* L. leucocephala. *Acid hydrolysis was carried out by mixing 0.25 ml of nodule extract with 1.0 ml of 6N HCl and heating at 110 C for 1 hr. GLNase and ASNase treatments were as described in Materials and Methods*

Amino acid	Untreated	Acid-hydrolyzed	GLNase-treated	ASNase-treated
		μmol/g fresh wt nodule (%)		
Asp	1.4 (3.6)	34.7 (43.5)	0.6 (2.3)	21.0 (37.0)
Asn/Gln	23.3 (60.1)	1.8 (2.3)	16.2 (62.5)	7.8 (13.8)
Glu	6.2 (16.0)	7.3 (9.1)	4.3 (16.6)	5.6 (9.9)
NH_4^+	7.9 (20.4)	36.0 (45.1)	4.8 (18.5)	22.3 (39.3)

present in leaves, stems, roots, and nodules from *L. leucocephala.* Three or four small unidentified peaks, which represented less than 0.1 μmol/g fresh weight, were evident in all chromatograms. In Table 2, as in subsequent tables, "not determined" indicates that the peak was too small for an accurate calculation of the area. Mimosine was the predominant free amino acid in the aboveground organs, followed by asparagine and γ-amino butyric acid (GABA). The level of ammonium ion was also relatively high in leaves. In the belowground organs, asparagine was the predominant free amino acid, followed by glutamic acid (in nodules only), mimosine, and NH_4^+ and GABA. Over half of the free amino acid content of the nodules was in asparagine + glutamine (which coeluted from the amino acid analyzer). Analysis of the relative amounts of aspartic acid and glutamic acid in extracts after being subjected to either acid hydrolysis or treatment with a specific deamidase demonstrated that asparagine was the predominant amide in the asparagine/glutamine/serine/threonine peak of untreated extracts (Table 3). Glutaminase had no real effect on the asparagine/glutamine/serine/threonine peak, whereas asparaginase had a major effect on that peak (Table 3).

In a study where the time course of change in free amino acid levels, plant height and weight, and acetylene reduction activity were followed, the only amino acids whose levels changed significantly during and after nodulation were aspartic acid, asparagine, glutamic acid, and the ammonium ion (Table 4). Correlation coefficients of linear regressions were calculated for each pair of parameters (Table 5); there were strong correlations between aspartic and glutamic acids ($r = 0.772$), asparagine and ammonia ($r = 0.875$), acetylene reduction and glutamic acid ($r = 0.899$), as well as between plant age and weight ($r = 0.958$) or height ($r = 0.895$). After 1 week (during which time the high level of asparagine is most prob-

TABLE 4. *Total free amino acid pools, plant height, plant fresh weight, and acetylene reduction activity of* L. leucocephala *trees at 1 to 7 weeks of age. Nodules first appeared on 3-week-old trees*

Amino acid	Weeks						
	1	2	3	4	5	6	7
				μmol/g fresh wt[a]			
Asp	0.83 ± 0.09	0.42 ± 0.03	0.65 ± 0.07	0.94 ± 0.09	1.10 ± 0.07	0.48 ± 0.07	0.49 ± 0.04
Asn	45.86 ± 4.60	8.81 ± 0.85	6.09 ± 0.78	11.50 ± 1.53	15.70 ± 1.73	10.67 ± 1.01	15.40 ± 2.01
Glu	0.68 ± 0.04	0.54 ± 0.02	1.19 ± 0.11	1.41 ± 0.08	1.79 ± 0.05	0.97 ± 0.03	0.88 ± 0.06
Ala	1.65 ± 0.09	1.26 ± 0.06	1.40 ± 0.04	1.53 ± 0.10	1.71 ± 0.06	1.46 ± 0.09	0.96 ± 0.05
Mim[c]	10.66 ± 0.64	8.49 ± 0.60	14.13 ± 0.53	11.08 ± 1.53	10.86 ± 1.31	5.89 ± 0.73	9.19 ± 0.90
GABA[d]	2.05 ± 0.14	2.22 ± 0.07	2.64 ± 0.23	2.59 ± 0.19	2.68 ± 0.08	2.29 ± 0.11	1.87 ± 0.10
His	1.68 ± 0.17	0.48 ± 0.02	0.40 ± 0.11	0.16 ± 0.02	0.22 ± 0.01	nd	0.13 ± 0.01
NH_4^+	17.58 ± 0.75	6.22 ± 0.44	7.49 ± 0.34	4.61 ± 0.33	5.42 ± 0.31	3.96 ± 0.79	3.31 ± 0.37
Arg	2.89 ± 0.94	0.13 ± 0.02	0.10 ± 0.01	nd	0.51 ± 0.16	0.45 ± 0.11	0.45 ± 0.16
Plant ht. (cm)	3.24 ± 0.37	7.14 ± 0.32	7.46 ± 0.49	9.26 ± 0.16	12.08 ± 0.49	16.96 ± 0.36	12.38 ± 0.35
Plant wt. (g)	0.33 ± 0.02	0.45 ± 0.03	0.44 ± 0.05	0.76 ± 0.05	0.97 ± 0.05	1.33 ± 0.13	1.23 ± 0.08
AR[e]	0	0	265.0	269.2	377.6	285.6	147.4

Pro, Gly, Val, Met, Leu, Tyr, Phe, and Lys contents never exceeded 1.0 μmol/g fresh wt.

[a] Mean ± SE of 5 samples.

[b] nd = not determined (see text).

[c] Mimosine.

[d] γ-Aminobutyric acid.

[e] Acetylene reduction (μmol ethylene/gfw nodule/hr).

TABLE 5. *Correlations between age, height and fresh weight, acetylene reduction activity, aspartate, asparagine, glutamate, and NH_4^+ levels of* L. leucocephala. *Correlations were calculated using data from Table 4*

	Plant weight	Plant height	NH_4^+	Glu	Asn	Asn[a]	Asp	AR
Weeks	0.958**	0.895**	−0.778*	0.367	−0.447	0.837*	−0.133	0.590
AR[b]	0.535	0.644	−0.565	0.899**	−0.500	0.590	0.463	
Asp	−0.149	−0.190	0.236	0.772**	0.308	0.069		
Asn	−0.335	−0.532	0.875**	−0.289				
Glu	0.286	0.340	−0.361			0.531		
NH_4^+	−0.699	−0.780*				−0.878**		
Height	0.947**					0.742		
Weight						0.763*		

[a] Correlations were calculated using μmol Asn/g fresh wt for week one as zero.
[b] Acetylene reduction.
* Significant at 0.05 probability level.
** Significant at 0.01 probability level.

ably seed-derived), a strong correlation was also found between asparagine and plant age ($r = 0.837$) and a strong inverse relationship between asparagine and NH_4^+ ($r = -0.878$).

The N content and ^{15}N enrichment of fractions from nodules exposed to $^{15}N_2$ are given in Table 6. As expected, the soluble amino acid fraction had the highest level of ^{15}N while lower, but significant, enrichment was observed in the pellet. The ureide fraction contained very little nitrogen with no measurable isotope enrichment.

In a more detailed study of the amino acid/amide fraction, free ammonia, aspartic acid, glutamic acid, asparagine, and glutamine were individually analyzed for their enrichment of ^{15}N. During the period of time studied, glutamic acid and asparagine were found to contain over 95% of the ^{15}N fixed into these compounds with the amino- and amide-N of asparagine accounting for over 75% (Table 7). Surprisingly, the amino-N of asparagine contained twice as much of the ^{15}N fixed as did the amide-N; likewise, the amino-N of glutamic acid and glutamine showed a greater ^{15}N enrichment than did the amide-N of glutamine.

DISCUSSION—The foliage of *L. leucocephala* has been reported to contain approximately 4% N (National Research Council, 1984). The percentages of N in most tissues of this plant are similar to those for other legumes (DuBois and Burris, 1986; Ohyama, 1983) with the exception of the nodules. Determinate type nodules generally contain 5–6% N (DuBois and Burris, 1986; Ohyama, 1983), but in this study we found an average of 7.2% N in the indeterminate nodules of *Leucaena*. The reason for this high percentage of N in these nodules is not known.

Mimosine has been reported to comprise up to 5% of the dry matter of *Leucaena* (National Research Council, 1984). In the study reported here, we found mimosine levels approaching 2.5% of the dry weight (assuming 80–90% water content in leaves). With the exception of mimosine (which appears to be unique to members of the Mimosidae), asparagine was the free amino acid in highest concentration in all four organs of *Leucaena* that we analyzed. The asparagine content ranged from 25% of the total free amino acid pool in leaves to 56% in the nodules. Although asparagine was routinely estimated as the total chromatographic peak which also included glutamine, serine, and threonine, it appeared to make up the majority of that peak (see Tables 3 and 7); the estimation made, therefore, is believed to be largely justified. Atkins et al. (1983) reported asparagine

TABLE 6. *N content and atom % ^{15}N of the insoluble pellet, ureide and total amino acid fractions of nodules from greenhouse-grown* L. leucocephala. *Nodules were exposed to $^{15}N_2$ for 1 hr prior to extraction*

Fraction	mg N/g fresh wt nodules		Atom % excess ^{15}N
	^{15}N exposed[a]	Control[b]	
	(mean ± SE)		(mean ± SE)
Pellet	6.0 ± 0.3	4.8 ± 0.6	0.0051 ± 0.0001
Ureide (× 10^3)	14.0 ± 2.0	12.0 ± 0.4	−0.0031 ± 0.0001
Amino acid	6.7 ± 0.4	4.4 ± 0.1	0.0670 ± 0.0009

[a] Mean of 6 samples
[b] Mean of 2 samples.

levels between 20 and 30% (molar basis) of the soluble nitrogen pool in leaves of *Lupinus albus* along with high levels of alanine and GABA. We also found the levels of the latter two amino acids to be higher than those for most other free amino acids in *Leucaena*.

The transport of recently fixed N from the nodules of *L. leucocephala* appears to occur via asparagine. The data (Table 6) also indicate that the level of free asparagine N in the nodules accounts for approximately half of the total N in this organ. Not only was the N content of the ureide fraction very small in comparison to other fractions of the nodules, but the ureide fraction showed no enrichment of isotope following the exposure of nodules to $^{15}N_2$. The negative value for atom % ^{15}N excess in the ureide fraction (Table 6) is very interesting. This negative value may represent some isotope fractionation in the metabolism of another nonacidic N compound (Kohl and Shearer, 1980). In contrast, the asparagine component of the nodules showed a substantial increase in ^{15}N concentration; over 75% of the newly fixed N was found in asparagine. In that experiment, NH_4^+ and the amide-N of asparagine showed ^{15}N enrichments lower than that of the amino-N of the acidic amino acids/amides, in contrast to what would be expected immediately following $^{15}N_2$ fixation. This may be accounted for in one of two ways. First, immediately after the incubation period with $^{15}N_2$, the nodules were exposed to air for a short period of time in order to determine their fresh weight. During this time, they might have fixed an amount of natural atmospheric N_2 which significantly chased the ^{15}N from NH_4^+ and asparagine and glutamine amide-N (Table 7). Secondly, these were relatively long-term experiments in terms of the initial assimilation of ^{15}N. In a few minutes after addition of $^{15}N_2$, the ^{15}N would go to ammonium and then to the amide groups of asparagine and glutamine, but with time would accumulate in the metabolically more stable amino position. Asparagine was not only found to be the common free amino acid in highest concentration, but the level of this amide (along with those of aspartic and glutamic acids) showed strong positive correlations with measurements of N_2 fixation (NH_4^+ content and acetylene reduction) during early development and nodulation. The low rates of N_2 fixation reported here (relative to those reported elsewhere; Hogberg and Kvarnstrom, 1982; National Research Council, 1984; van Kessel et al., 1983) are most likely due to the use of excised nodules, rather than intact nodulated plants. However, the determination of N_2 fixation rates was not an objective of this study. Our objective was to assess where (into which compound) the fixed N_2 went. Therefore, the use of excised nodules was justified.

TABLE 7. *Incorporation of ^{15}N into ammonia and amino acids by excised nodules of* L. leucocephala *exposed to ^{15}N-enriched N_2. Nodules were exposed to $^{15}N_2$ for 1.5 hr prior to extraction. The data are means of 3 samples*

Fraction	Total N	Atom % excess ^{15}N	^{15}N	%[a]
	(μmol)		(nmol)	
Free NH_4^+	2.3	0.0016	0.04	0.1
Asp amino-N	1.2	0.0811	0.9	2.1
Glu amino-N	3.0	0.3176	9.6	20.1
Asn amide-N	24.1	0.0472	11.4	23.8
Asn amino-N	24.1	0.1052	25.4	53.1
Gln amide-N	0.6	0.0188	0.1	0.2
Gln amino-N	0.6	0.0501	0.3	0.6

[a] nmoles ^{15}N/47.8 (total nmoles ^{15}N) × 100.

Evidence presented here shows that *L. leucocephala* assimilates its newly fixed N_2 into asparagine in the nodule. This plant not only forms high levels of asparagine in the nodules (27.3 μmol/g fresh wt) as compared to the ureide transporting soybean (0.14 μmol/g fresh wt; Streeter, 1987), but over 75% of the newly fixed ^{15}N is accounted for in this specific amino acid (see Table 7). Also, following nodulation and depletion of seed-derived N, there is a strong correlation between plant age and asparagine content. Therefore, all evidence indicates that in *L. leucocephala*, asparagine is a primary sink for fixed N_2 and that fixed N_2 is probably exported from the nodule in this form.

The tissues of *L. leucocephala* have N levels that are quite similar to those of other legumes. However, the rates of nitrogen fixation activity for this legume are relatively very high. An understanding of the modes for nitrogen assimilation and transport in such productive native plants could be of help in attempts to improve N_2 fixation and the assimilation of fixed N_2 in domesticated legumes.

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