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Amino Acid Transport and Metabolism in Relation to the Nitrogen Economy of a Legume Leaf[1]

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ABSTRACT

Net balances of amino acids were constructed for stages of development of a leaf of white lupin (*Lupinus albus* L.) using data on the N economy of the leaf, its exchanges of amino acids through xylem and phloem, and net changes in its soluble and protein-bound amino acids. Asparagine, aspartate, and γ-aminobutyrate were delivered to the leaf in excess of amounts consumed in growth and/or phloem export. Glutamine was supplied in excess until full leaf expansion (20 days) but was later synthesized in large amounts in association with mobilization of N from the leaf. Net requirements for glutamate, threonine, serine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine were met mainly or entirely by synthesis within the leaf. Amides furnished the bulk of the N for amino acid synthesis, asparagine providing from 24 to 68%. *In vitro* activity of asparaginase (EC 3.5.1.1) exceeded that of asparagine:pyruvate aminotransferase (EC 2.6.1.14) during early leaf expansion, when *in vivo* estimates of asparagine metabolism were highest. Thereafter, aminotransferase activity greatly exceeded that of asparaginase. Rates of activity of one or both asparagine-utilizing enzymes exceeded estimated rates of asparagine catabolism throughout leaf development. *In vitro* activities of glutamine synthetase (EC 6.3.1.2) and glutamate synthase (EC 1.4.7.1) were consistently much higher than that of glutamate dehydrogenase (EC 1.4.1.3), and activities of the former two enzymes more than accounted for estimated rates of ammonia release in photorespiration and deamidation of asparagine.

A recent paper (15), studying the economy of C and N in the life of a leaf of white lupin (*Lupinus albus* L.), has depicted transport exchanges in xylem and phloem as key components in the growth and mature functioning of the leaf and in the integration of its activities with the rest of the plant. The present paper combines this information with data on amino acid composition of the phloem and xylem streams and of the insoluble and soluble pools of the leaf, to construct balance sheets for import, export, and metabolism of specific amino compounds during different stages of leaf development, and to match this information against *in vitro* studies of enzymes likely to be important in the N metabolism of the leaf. In view of the dominant role of asparagine (Asn) in the transport and metabolism of N in white lupin (1, 2, 16-18) and other legume species (22), special study is made of the involvement of this amide in the overall N metabolism of the leaf.

MATERIALS AND METHODS

Plant Material. Effectively nodulated (*Rhizobium* strain WU425) white lupin (*Lupinus albus* L. cv Ultra) plants were grown in minus N sand culture in a naturally lighted glasshouse during July to October in Perth, Western Australia. Leaflets of different age were collected (30 leaves at each time of sampling) from the uppermost main stem leaf, using plants identical to those utilized in a companion paper (15).

Enzyme Assays. Leaflet samples (subsamples of 10 leaflets) were thoroughly homogenized in a chilled mortar and pestle with extraction buffer (2-3 cm^3/g fresh tissue) containing 30 mM Tricine-KOH (pH 8.0), 20 mM $MgCl_2$ and 2 mM DTT. The homogenate was centrifuged (25,000*g* for 10 min at 4°C, and the supernatant was used as the source of enzyme.

GS[3] (EC 6.3.1.2) was assayed in a biosynthetic reaction based on glutamyl hydroxamate synthesis (13), GDH (EC 1.4.1.3) as ammonia-dependent NADH oxidation (5). GOGAT (EC 1.4.7.1) was measured using an assay mixture with methyl viologen/dithionite as electron donor (23). The reaction was terminated after 20 min by vigorously vortexing the assay tubes and boiling for 1 min. Precipitated protein was removed by centrifugation, and the supernatant was added to a Dowex — 1 × 10 formate-form column (9 × 20 mm). Following elution with 6 ml water to remove Gln, Glu was eluted with 3 ml 2 M HCOOH (10) and assayed with ninhydrin (24). Tissue extracts for the GS, GDH, and GOGAT assays were desalted by passage through Sephadex G-25 before estimating enzyme activity.

Preparation of asparagine:pyruvate aminotransferase (EC 2.6.1.14) for assay required treatment of crude extracts with polyethylene glycol 6000 (4). After centrifugation, the resultant pellets were resuspended in homogenization buffer and assayed by measuring the production of alanine and/or 2-ketosuccinamic acid from L-asparagine and sodium pyruvate (4).

Asparaginase (EC 3.5.1.1) was precipitated with 55% ammonium sulfate prior to assay, and activity of the resuspended pellets was determined by the formation of aspartate from asparagine in the presence of high levels of KCl (4).

Asparagine solutions were routinely passed through ion exchange resin (Dowex 1, formate form) at pH 6.5 to remove contaminating aspartic acid before use in assay.

Activities of all enzymes were determined at 30°C.

Collection and Analysis of Transport Fluids Serving the Leaf. Samples of upper stem tracheal (xylem) sap and petiole phloem sap were collected and assayed for nitrogenous solutes as described previously using a Beckman 118C Amino Acid Analyzer (physiological fluids mode) (1, 2, 16). Fifteen plants were used at each time of sampling (15).

Determination of Soluble Amino Compounds in Leaf Tissue. Leaflets (subsample of 20 leaves) were extracted with 80% (v/v)

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[3] Abbreviations: GS, glutamine synthetase; GDH, glutamate dehydrogenase; GOGAT, glutamine oxoglutarate amidotransferase.

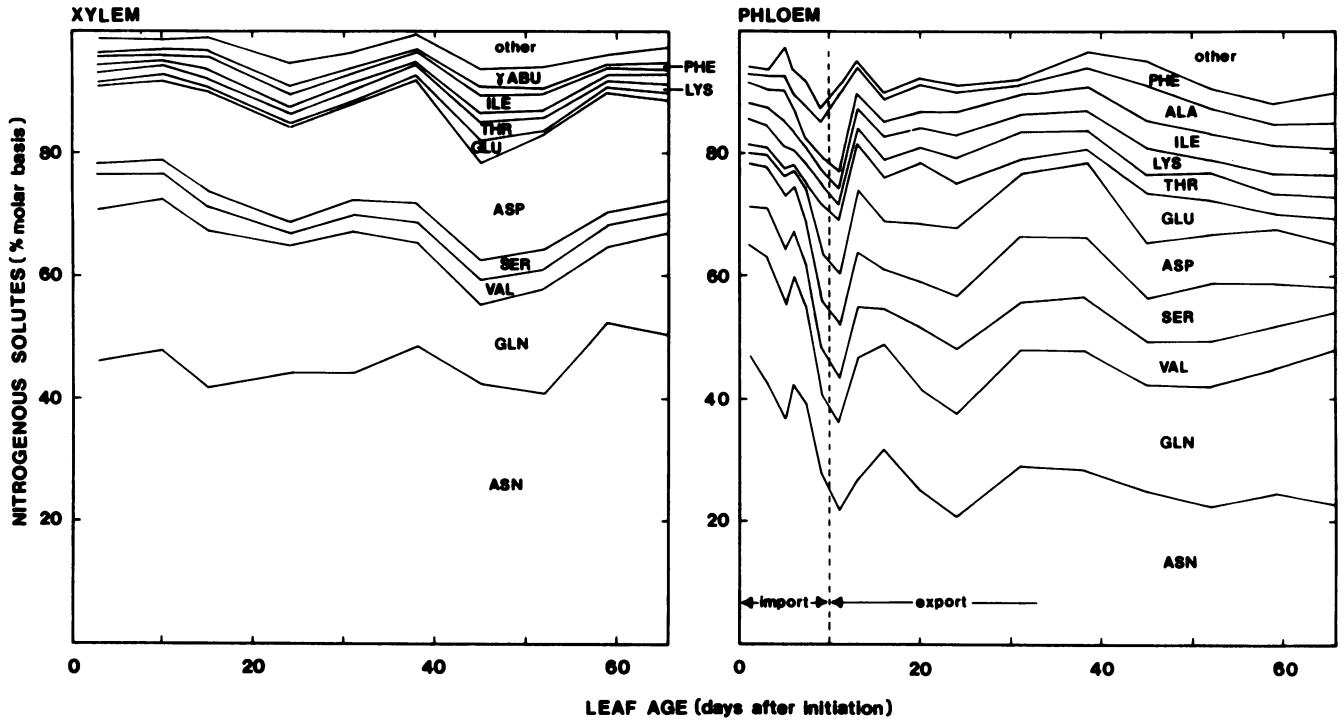

FIG. 1. Changes with time in the composition of amino acids (% molar basis) in upper stem tracheal (xylem) sap and phloem exudate collected from the petioles of the uppermost main stem leaf of effectively nodulated plants of *L. albus*. The phases of import and export through phloem were those established previously for this leaf (15).

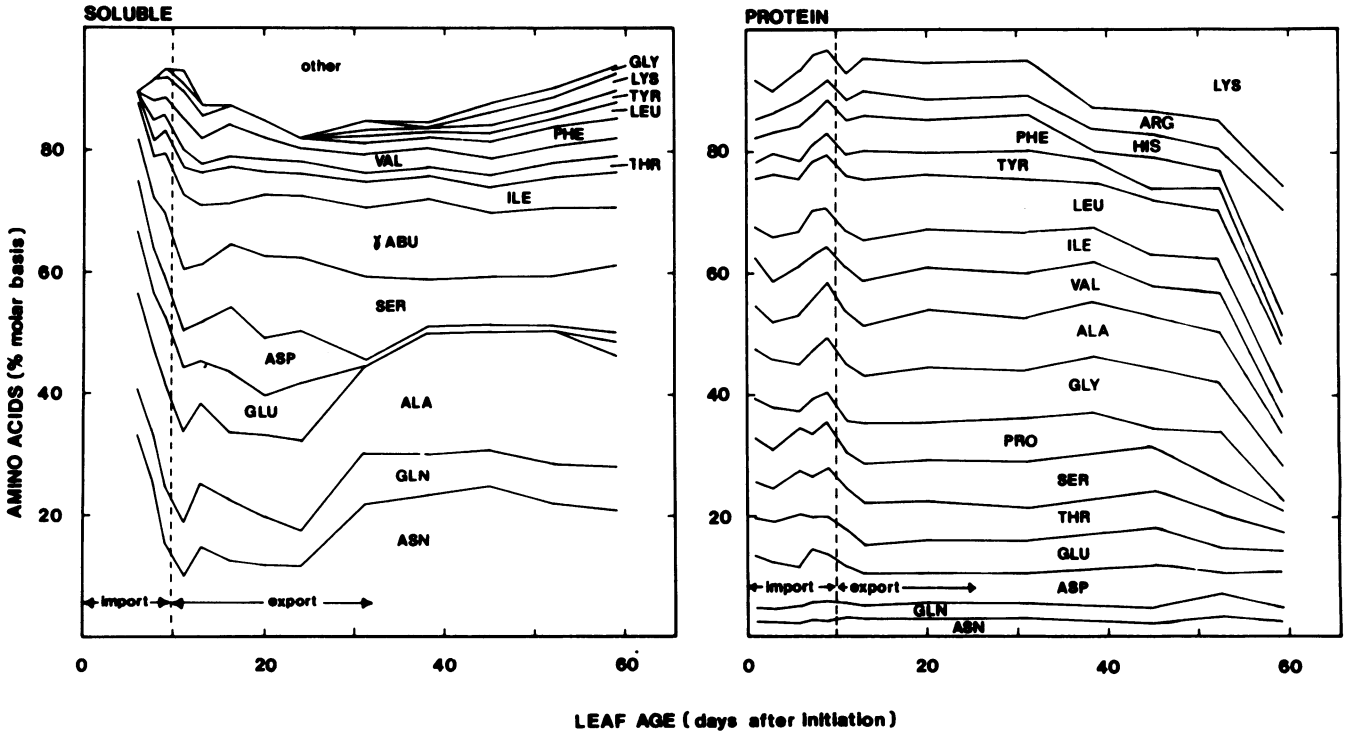

FIG. 2. Changes with leaf age in the composition of amino acids (% molar basis) in the soluble nitrogen pool and in the protein of leaflets of the upper main stem leaf of effectively nodulated plants of *L. albus*. The phases of import and export through phloem were those established previously (15).

ethanol and the water-soluble compounds recovered and analyzed for amino acids using the Amino Acid Analyzer (1, 17).

Determination of Amino Acids in Leaf Protein. Samples for hydrolysis of protein comprised either the insoluble material from leaflets following extraction with 80% (v/v) ethanol, or aliquots of extracts of leaflets prepared for enzyme assay (see above) and desalted using Sephadex G-25. Paired samples of an extract were hydrolyzed for 48 h in 6 M HCl at 110°C, one sample with, the other without, prior treatment with bis(1,1-trifluoracetoxy)-iodobenzene (BTI) (for preparation, see Ref. 14), to convert

Table I. *Balance Sheets for Transport Exchanges and Utilization of Amino Acids over the Period 1 to 11 Days after Initiation of the Top Main Stem Leaf of L. albus L.*

This stage of development ended with the leaf one-third expanded. Net import through phloem continued throughout the 11 d (see 15).

Amino Acid	1. Input by Xylem	2. Input by Phloem	3. Net Gain as Leaf Soluble + Protein	4. Net Balance of Leaf (Surplus +) (Deficit −) ([1 + 2] − 3)	5. Proportion of Net Deficit Met by Synthesis in Leaf ([4 ÷ 3] × 100)
		μmol/leaf[a]			%
Asn	36.43	5.00	3.65	37.78	
Gln	18.58	3.22	4.61	17.19	
Asp	9.29	2.15	9.62	1.82	
Glu	ND[b]	2.15	12.85	−10.70	84
Thr	0.72	0.72	10.29	−8.85	86
Ser	1.43	2.15	10.29	−6.71	66
Pro	ND	ND	3.23	−3.23	100
Gly	ND	0.72	11.51	−10.79	94
Ala	ND	2.86	12.42	−9.56	77
Val	2.86	2.15	11.40	−6.39	56
Ile	1.43	0.72	8.28	−6.13	74
Leu	ND	ND	12.29	−12.29	100
Tyr	ND	ND	4.17	−4.17	100
Phe	ND	0.72	8.31	−7.59	92
γAbu	1.43	0.72	0.76	1.39	
His	0.72	ND	5.61	−4.89	88
Lys	ND	0.48	10.10	−9.62	96
Arg	ND	ND	6.74	−6.74	100

[a] Estimates derived from net balance of N for leaf (Ref. 15, Fig. 4) and amino acid composition data of Figures 1 and 2 (this paper).

[b] ND, not detectable.

carboxamide residues to the corresponding amines (20). The differences between Asp and Glu levels of hydrolysates with and without BTI treatment were assumed to provide measures of Asn and Gln residues in the protein samples. The accuracy of this method was tested using a commercial sample of ribonuclease A (Sigma). Asn and Gln values estimated by the BTI method were found to agree closely with published values for amide content of the protein based on enzymic digestion (3).

Values for the proportion of aspartyl residues as Asp and Asn and glutamyl residues as Glu and Gln in the leaf hydrolysates were then matched against full analyses of other amino acids in the hydrolysates (no BTI treatment) to determine the total molar balance of amino acid residues in the protein.

RESULTS AND DISCUSSION

Amino Acid Balance of Transport Fluids and the Soluble and Insoluble N Fractions of the Leaf. The composition of amino acids in xylem (upper stem, tracheal) sap supplying the leaf and in phloem sap collected from the petiole of the study leaf was as shown in Figure 1. Xylem contained a mixture of amino compounds which varied only slightly in relative composition during the 66-d study period. As in earlier studies on *L. albus* (2, 16, 17), Asn, Gln, and Asp were the predominant forms of N in xylem. Phloem sap composition, though varying greatly in sugar:amino acid balance, and hence in C:N ratio (see companion paper [15]), had a relatively constant balance of amino compounds as the leaf changed from importing through the phloem (1–11 d) to later stages of growth (11–24 d), maturity, and senescence (24–66 d) when net export was recorded through phloem (15). There was, however, a progressive decline in phloem content of Asn relative to other amino acids during the importing phase (1–11 d) and a slight rise in Gln relative to Asn during the later stages of leaf development.

Ethanol-soluble N was less than 10% of the total N of the leaf (15), with 80% or more of this soluble N as amino acids. Compositional changes in soluble amino acids with leaf age (Fig. 2) were greater than noted above for transport fluids. There was an early decline, followed by a rise in relative content of Asn, a sharp decline in Asp content at 30 d, and a tendency for the ratio of Asn:Gln to be much lower during late leaf expansion (13–30 d) than earlier or later in leaf development.

The amino acid composition of leaf protein (Fig. 2) was relatively constant up to 30 d, despite massive increases in total leaf N during this phase. Thereafter (days 30–66), as total leaf N remained constant or was decreasing (15), the protein fraction became progressively elevated in relative contents of the basic amino acids Lys and His. At all stages of leaf development, the amounts of Glu and Asp residues in protein exceeded those of Gln and Asn, respectively.

Amino Acid Balance of the Leaf at Different Stages of Development. The same four developmental stages were studied as in the companion paper (15); *viz.* phase 1 (1–11 d), early growth with net import through phloem; phase 2 (11–20 d), later growth with increasing net export through phloem; phase 3 (20–38 d), maturity with high rates of phloem export, little change in total N, but some loss of Chl and photosynthetic activity; with phase 4 (38–66 d), later maturity to early senescence, with continuing phloem export, further losses of photosynthetic activity, and a significant decline in leaf N.

The balance for each amino acid was determined using values for net import of N in xylem and export or import in phloem, net changes in soluble and insoluble N fractions of the leaf (all values from the companion paper [15]), and data for amino acid composition of xylem and phloem sap (Fig. 1) and of the soluble and

Table II. *Balance Sheets for Transport Exchanges and Utilization of Amino Acids over the Period 11 to 20 Days after Initiation of the Top-Main Stem Leaf of L. albus L.*

This stage of development ended with the leaf fully expanded. Net export through phloem occurred continuously (15).

Amino Acid	1. Input by Xylem	2. Output by Phloem	3. Net Gain as Leaf Soluble + Protein	4. Net Balance of Leaf (Surplus +) (Deficit −) (1 − [2 + 3])	5. Proportion of Net Deficit Met by Synthesis in Leaf (4 ÷ [2 + 3] × 100)
		μmol/leaf[a]			%
Asn	102.15	13.22	6.60	82.33	
Gln	61.79	8.93	4.49	48.37	
Asp	38.58	5.00	8.70	24.88	
Glu	ND[b]	5.00	7.95	−12.95	100
Thr	2.86	1.43	7.64	−6.21	69
Ser	5.00	4.29	11.16	−10.45	68
Pro	ND	ND	7.67	−7.67	100
Gly	ND	1.43	9.82	−11.25	100
Ala	ND	2.86	12.53	−15.39	100
Val	8.58	5.72	9.43	−6.57	44
Ile	3.58	1.43	9.75	−7.60	68
Leu	1.43	ND	11.90	−10.47	88
Tyr	ND	ND	5.41	−5.41	100
Phe	1.43	ND	6.88	−5.45	80
γAbu	6.43	ND	2.05	4.38	
His	2.15	ND	4.19	−2.04	49
Lys	1.67	1.19	5.63	−5.15	76
Arg	ND	ND	6.83	−6.83	100

[a] Estimates derived from net balance of N for leaf (Ref. 15, Fig. 4) amino acid composition data of Figures 1 and 2 (this paper).

[b] ND, not detectable.

protein pools of the leaf (Fig. 2). Combining these data, as shown in Tables I to IV, it was possible to estimate the extent to which the net demand for each amino acid by the leaf in growth and/or phloem transport might have been met by import of the same amino acid through the parent plant, as opposed to synthesis within the tissues of the leaf.

In establishing the net balance of an amino compound during a specific interval of leaf development (Tables I–IV, column 4), it was assumed that where the amounts of the compound increased in the soluble pool and protein of the leaf (Tables I and II, column 3), these increases had been met to the maximum possible extent by direct incorporation of the compound from the entering phloem and xylem streams (Table I) or xylem stream (Table II). Similarly, where phloem export was occurring (Tables II–IV, column 2), it was assumed that the export process had maximum possible access to the compound in question by direct transfer from the entering xylem streams (Tables II–IV) and from any declining pools of soluble amino acids or protein in the leaf (Tables II and IV). On this basis, all values for proportional synthesis of a compound by the leaf (Tables I–IV, column 5) were clearly minimum estimates of the leaf's true synthetic capacity.

During the initial 11 d of leaf growth (Table I), the three major amino acids of xylem and phloem (Asn, Gln, and Asp) and γ-Abu were delivered in excess of the amounts of the same compounds incorporated into the soluble and protein pools of the leaf. For all other amino compounds, amounts imported were less than amounts incorporated by the leaf, indicating that synthesis of the compounds in question must have occurred in the leaf, presumably involving a utilization of the N delivered in excess as amides and Asp. The extent to which synthesis in the leaf met the leaf's requirement for a specific amino acid varied from 56% for Val to up to 100% for Pro, Leu, Tyr, and Arg. The latter four compounds were not supplied in detectable amounts in either xylem or phloem. The data of Table I indicated that 113 μmol extra amino and amide groups were available for amino acid synthesis through xylem and phloem import during the 11-d period (see positive values in Table I, column 4) while the negative values recorded in the same column of the Table indicated that an almost matching number of amino acids (108 μmol) had been required for synthesis of compounds not available, or inadequately supplied from the parent plant.

In the second phase of growth (11–20 d, Table II), the situation was complicated by increasing export of amino acids in phloem, despite continued heavy demand for N in leaf growth. As in phase 1, major solutes of xylem (Asn, Gln, Asp, γ-Abu) were delivered in excess, and, of the remaining amino compounds, net balances (Table II, column 4) indicated that some, not supplied in detectable amounts in xylem (Glu, Pro, Gly, Ala, Tyr, and Arg), were synthesized completely by the leaf while the demand for the others was met partly by import, partly by synthesis. Significantly, many of the amino acids formed completely in the leaf were those identified previously as the major amino acid products of photosynthetic carbon fixation in legume leaves (18). The match between the number of excess amino and amide groups imported in xylem (291 μmol/leaf) and the apparent requirement for amino nitrogen to meet leaf growth and phloem export (148 μmol) was not so close as in phase 1. Presumably, the excess of supply over demand reflected a diversion of N to the formation of nitrogenous constituents other than amino acids within the leaf.

During the final two stages of development (phases 3 and 4), net losses occurred in total amounts of virtually all amino acids in soluble and protein pools (Tables III and IV, column 3), the only

Table III. *Balance Sheets for Transport Exchanges and Utilization of Amino Acids over the Period 20 to 38 Days after Initiation of the Top Main Stem Leaf of L. albus L.*

This stage of development represented the early mature phase of leaf functioning, recording maximum rates of photosynthesis and translocation (see 15).

Amino Acid	1. Input by Xylem	2. Output by Phloem	3. Net Gain (+) or Loss (−) as Leaf Soluble + Protein	4. Net Balance of Leaf (Excess +) (Deficit −) (1 − [2 + 3])	5. Proportion of Net Deficit Met by Synthesis in Leaf (4 ÷ [2 + 3] × 100)
			μmol/leaf[a]		%
Asn	69.29	62.15	0.43	6.71	
Gln	36.08	41.43	−2.02	−3.33	9
Asp	24.29	27.15	−5.24	2.38	
Glu	ND[b]	ND	−4.89	4.89	
Thr	1.43	7.15	−3.61	−2.11	60
Ser	2.86	21.43	−5.94	−12.63	82
Pro	ND	ND	−4.03	4.03	
Gly	ND	ND	−4.16	4.16	
Ala	ND	7.15	−3.70	−3.45	100
Val	4.29	19.29	−4.49	−10.51	71
Ile	2.15	10.00	−4.64	−3.21	60
Leu	1.43	2.86	−4.85	3.42	
Tyr	ND	ND	−2.17	2.17	
Phe	0.72	8.58	−2.94	−4.92	88
γAbu	3.58	ND	0.68	2.90	
His	1.43	ND	−1.68	3.11	
Lys	1.19	4.29	−2.46	−0.64	35
Arg	ND	ND	−3.30	3.30	

[a] Estimates derived from net balance of N for leaf (Ref. 15, Fig. 4) and amino acid composition data of Figures 1 and 2 (this paper).

[b] ND, not detectable.

exceptions to this being Asn in phase 3, Pro in phase 4, and γ-Abu during both phases 3 and 4. A number of amino acids were in negative balance (see deficits in column 4), indicating a supply in xylem and loss from the soluble and protein pools of the leaf in amounts lesser than export in phloem. The data indicated a net import of 60 μmol excess amino plus amide groups in phase 3, 181 μmol in phase 4, and that, of these amounts of amino N, 45 μmol (phase 3) and 185 μmol (phase 4) were utilized for amino acid synthesis within the leaf. The balance sheets thus indicated a close match between supply and demand of amino N at these stages of development.

As in earlier stages, compounds delivered to the leaf in excess of demand during phases 3 and 4 were Asn, Asp, and γ-Abu, whereas the proportions of the net deficits of other amino acids met by synthesis in the leaf varied markedly from one compound to another (Tables III and IV, column 5). However, in contrast to the situation in phases 1 and 2, the leaf was in negative balance for Gln, and the substantial export of this amide in phase 4 appeared to have been met principally by synthesis within the leaf. A previous study on *L. albus* (17) showed that the phloem stream translocated from old leaflets at the base of the shoot contained proportionately more N as Gln than did younger leaves closer to the shoot apex. These lower leaves, like the leaf during phase 4 of the present study, were in negative balance for N, so it may be typical of white lupin for senescing leaves mobilizing N to produce significant amounts of Gln during degradation of protein.

Using the data of Tables I to IV, it was possible to estimate the rate (μmol/d·leaf) at which Asn was metabolized *in vivo* by comparing the leaf's demands for this amide as sorted free Asn, as protein-bound Asn, and as Asn exported in phloem with the total import of Asn at various times throughout development. It was assumed, as indicated above, that 92% of the xylem to phloem transfer of N derived from xylem-borne asparagine involved direct transfer of unmetabolized amide. The resulting balance sheet for Asn (Table V) indicated that throughout the major period of leaf growth (1–15 d), the amounts of Asn metabolized were equivalent to 89 to 93% of the Asn imported by the leaf. Rates of Asn metabolism rose sharply over the first 6 d of leaf growth, reaching a peak of 17 μmol Asn/d·leaf during the interval 13 to 17 d, coincident with most rapid expansion of the leaf and highest rates of incorporation of N into leaf matter. Utilization rates then declined sharply (17–29 d), then rose again slightly as the leaf commenced to lose much of its N and Chl (15).

By expressing the data of Table V on a unit fresh weight basis, it was possible to compare estimated *in vivo* rates of Asn breakdown during leaf development with *in vitro* measurements of activities of the asparaginase and asparagine:pyruvate aminotransferase systems of the leaf. The comparisons (Fig. 3) indicated that during early growth (1–11 d), when rates of utilization per unit weight of leaf reached a peak, asparaginase activities were closely commensurate with, or significantly greater than, *in vivo* utilization of Asn. Aminotransferase activity, by contrast, remained low. After 15 d, the situation changed radically, activity of the aminotransferase system increasing greatly to levels several times higher than that of asparaginase, and *in vitro* activities of both enzymes increasing to levels each several times that of *in vivo* Asn utilization. This situation continued until 50 d, after which asparaginase activity declined to levels significantly below the *in vivo* estimates of Asn utilization, while the aminotransferase, though falling, still exhibited an activity 15 to 20 times that of *in vivo* utilization of Asn.

Although the affinity of the above two enzymes for Asn was

Table IV. *Balance Sheets for Transport Exchanges and Utilization of Amino Acids over the Period 38 to 66 Days after Initiation of the Top Main Stem Leaf of L. albus L.*

This stage showed losses of photosynthetic activity, Chl and total leaf N and declining export through phloem (see 15).

Amino Acid	1. Input by Xylem	2. Output by Phloem	3. Net Gain (+) or Loss (−) as Leaf Soluble + Protein	4. Net Balance of Leaf (Surplus +) (Deficit −) (1 − [2 + 3])	5. Proportion of Net Deficit Met by Synthesis in Leaf (4 ÷ [2 + 3] × 100)
		μmol/leaf[a]			%
Asn	153.93	106.43	−3.56	51.06	
Gln	36.43	86.43	−2.79	−47.21	56
Asp	45.00	37.15	−5.23	13.08	
Glu	ND[b]	12.86	−4.34	−8.52	100
Thr	3.58	14.29	−5.04	−5.67	61
Ser	5.00	30.72	−5.15	−20.57	80
Pro	ND	ND	4.71	−4.71	100
Gly	ND	ND	−8.24	8.24	
Ala	3.58	12.86	−8.45	−0.83	19
Val	5.72	30.00	−5.27	−19.01	77
Ile	2.86	17.86	−4.23	−10.77	79
Leu	1.43	9.29	−7.71	−0.15	9
Tyr	0.72	7.86	−2.58	−4.56	86
Phe	0.72	14.29	−2.91	−10.66	94
γAbu	2.15	4.29	1.29	−3.43	61
His	1.43	4.53	−7.00	3.9	
Lys	1.43	8.10	−16.30	9.63	
Arg	ND	ND	−4.46	4.46	

[a] Estimates derived from net balance of N for leaf (Ref. 15, Fig. 4) and amino acid composition data of Figures 1 and 2 (this paper).

[b] ND, not detectable.

Table V. *Balance Sheets for Transport Exchanges and Metabolism of Asparagine during Different Stages of Development of the Top Main Stem Leaf of White Lupin (L. albus L.)*

Interval after Initiation	Total Import of Asn by Leaf[a]	Estimated Phloem Export as Xylem to Phloem Transfer of Asn[b]	Net Change in Leaf Soluble Asn[c]	Net Change in Leaf Protein Asn[c]	Estimated Utilization of Asn in Leaf Metabolism[d]	Proportion of Imported Asn Metabolized in Leaf[e]
d			*μmol/leaf·d*			%
1–5	1.59	0	0.08	0.09	1.42	89
5–9	6.53	0	0.21	0.21	6.11	94
9–13	7.65	0.16	0.24	0.20	7.05	92
13–17	18.18	1.31	0.05	−0.12	16.94	93
17–21	7.74	2.11	0.42	0.24	4.97	64
21–25	3.61	2.87	−0.04	0.43	0.35	10
25–29	3.38	2.90	0.26	−0.12	0.34	10
29–39	4.97	3.01	0.02	−0.12	2.06	41
39–49	3.59	2.53	0.14	−0.18	1.10	31
49–66	6.38	3.49	−0.11	−0.03	3.03	47

[a] Import in xylem and phloem for first 11 d, in xylem only after 11 d. Data from accompanying paper (15) and Figure 1.

[b] Assumes that 92% of Asn of phloem translocate derived by xylem to phloem transfer of unmetabolized Asn (see 1).

[c] Data from sol N and insol N values of companion paper (15) and amino acid analyses of Figure 2.

[d] Derived as Asn import − (Asn phloem export [if occurring] + net change in sol and insol Asn in leaf).

[e] Asn utilized ÷ Asn imported × 100.

not determined in the present study of lupin leaves, K_m values for pea leaves were recorded as 8 mM (Asn) for asparaginase, 4.5 mM for the aminotransferase (4), and closely similar K_m values have been described for the asparaginase of developing lupin embryos (2) and maize endosperm (12). On the basis of inhibitor studies on the two Asn-utilizing enzymes during early leaf growth in pea, it was suggested (4) that, while both enzymes might be involved in Asn catabolism, the aminotransferase was the more likely major route for asparagine utilization (4). Labeling studies demonstrating extensive metabolism of Asn to form 2-hydroxysuccinamic acid (7, 21) also supported an involvement of the aminotransferase system in Asn breakdown in young pea leaves.

Table VI. *Estimated Mean Rates of NH_3 Release in Photorespiration and Asn Deamidation and Mean In Vitro Activities of GS and GOGAT for Different Phases[a] of Development of the Top Main Stem Leaf of White Lupin (L. albus L.)*

	Phase 1 (0–11 d)	Phase 2 (11–20 d)	Phase 3 (20–38 d)	Phase 4 (38–66 d)
		μmol/min·g fresh wt		
1. Net photoperiod fixation of CO_2[b]	1,024	4,096	3,036	1,596
2. Ammonia release in photorespiration[c]	256	1,024	759	399
3. Ammonia release from Asn deamidation[d]	56	29	1	4
4. *In vitro* GS activity	955	1,385	1,283	1,333
5. *In vitro* GOGAT activity	480	1,141	1,230	1,527

[a] Phases of leaf development as defined in companion paper (15) and legends to Tables I to IV.

[b] Data from Figure 2 of companion paper (15).

[c] Assumes rate of CO_2 release in photorespiration is equivalent to 25% of net photoperiod CO_2 fixation (9,19) and that 1 mol NH_2 is generated/mol CO_2 released (6,8).

[d] Derived from Asn metabolism data of Table V.

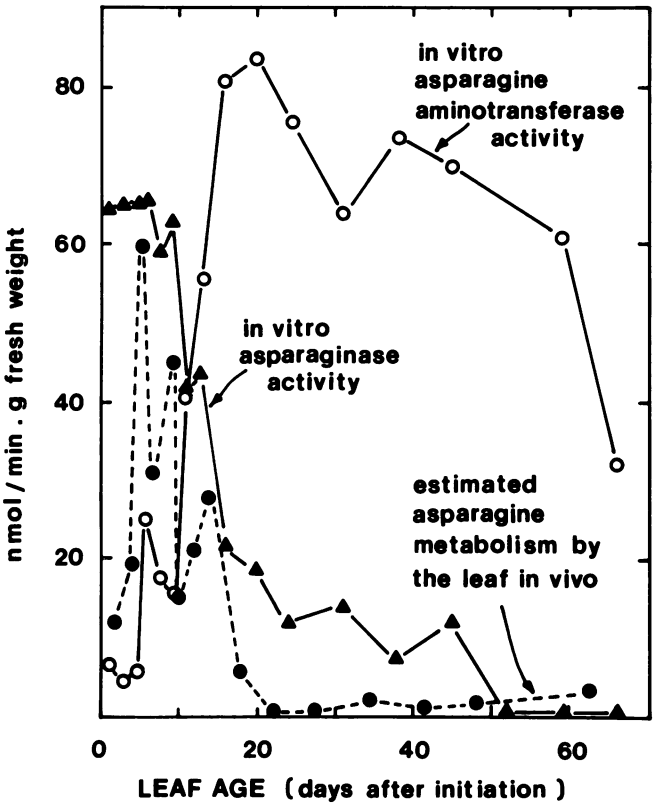

FIG. 3. Comparison of the estimated *in vivo* rates of Asn metabolism (taken from Table V) with the *in vitro* activities of two Asn-utilizing enzymes of leaflets of the uppermost main stem leaf of *L. albus*. The comparison extends from initiation to early senescence of the leaf.

Regardless of whether ammonia was being released through the action of asparaginase or from deamidation following an amino transfer reaction, the developing lupin leaf would obviously require effective mechanism(s) for utilizing ammonia. Assays showed that leaflet tissues had high *in vitro* activities for GS (0.55–1.65 μmol/min·g fresh weight) which were matched, in all but the initial 6 d of growth, by an equivalent rate of activity of GOGAT. By contrast, GDH activity remained at a very low level throughout development (0.02–0.05 μmol/min·g fresh weight) suggesting that, as in other leaves (11), a 'high affinity' pathway linked to photosynthetic activity was the route for ammonia reassimilation (11).

On present evidence, the most likely major source of ammonia-requiring assimilation of the leaf would be from the metabolism of Gly to Ser by mitochondria in photorespiration (6, 8). Values for the flux of C through the glycolate pathway were not available for lupin leaves; but, assuming that the mean flux was equivalent to 25% of the rate of net photoperiod CO_2 uptake (see data for leaves of other C_3 species [9, 19]), the likely rate of ammonia release in association with photorespiration was estimated, using data for net photoperiod uptake of CO_2 detailed in the companion paper (15). The estimates (Table VI) suggested that production of ammonia in photorespiration of the leaf (item 2) would have greatly exceeded rates of release of amide N from Asn utilization, but that the *in vitro* activities of GS and GOGAT were fully adequate for assimilating all of the ammonia generated in photorespiration and breakdown of amides entering leaf metabolism.

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