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Uptake and Utilization of Xylem-borne Amino Compounds by Shoot Organs of a Legume[1]

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ABSTRACT

Amino compounds representative of the major N solutes of xylem sap were pulse-fed (10 to 20 minutes) singly in ^{14}C-labeled form to cut transpiring shoots of white lupin (*Lupinus albus* L.). ^{14}C distribution was studied by autoradiography and radioassays of phloem sap, leaflet tissues, and shoot parts harvested at intervals after labeling. Primary distribution of N by xylem was simulated using a 20-minute labeling pulse followed by a 30-minute chase in unlabeled xylem sap. Shoots fed ^{14}C-labeled asparagine, glutamine, valine, serine, or arginine showed intense labeling of leaflet veins and marked retention (35 to 78%) of ^{14}C by stem + petioles. Shoots fed ^{14}C-labeled aspartic acid or glutamic acid showed heaviest ^{14}C accumulation in interveinal regions of leaflets and low uptake (11 to 20%) of ^{14}C by stem + petioles. Departing leaf traces were major sites of uptake of all amino compounds, and the implications of this were evaluated. Fruits acquired only 1 to 5% of the fed label directly from xylem, but more than doubled their intake during the period 30 to 160 minutes after feeding through receipt of ^{14}C transferred from xylem to phloem in stem and leaves. ^{14}C-Labeled asparagine and valine transferred directly from xylem to phloem, but the ^{14}C of ^{14}C-labeled aspartic acid and arginine appeared in phloem mainly as metabolic products of the fed compound. The labeling of the soluble pool of leaflets reflected these differences. The significance of heterogeneity in distribution and metabolism of xylem amino compounds in the shoot was discussed.

The xylem stream of legumes carries a complex mixture of nitrogenous solutes which, in symbiotically effective plants, results principally from the export of fixed N by root nodules (9, 11). One or more N-rich compounds (*e.g.* Asn, Gln, substituted amides, ureides) predominate in xylem when N_2 is being assimilated (7) and the significance of these in temporary storage of N or as sources of N for developing fruits has been evaluated (1, 4, 12). This paper examines the patterns of distribution and utilization of the major xylem amino compounds within variously aged shoots of the legume *Lupinus albus* L., defining the involvement of different organs and tissues in the uptake and metabolism of specific solutes from the xylem stream. The study complements earlier investigations (11, 15) on the significance of xylem to phloem transfer in the N nutrition of fruits of the species.

MATERIALS AND METHODS

Plant Material. Effectively nodulated plants of white lupin *L. albus* cv. Ultra) were grown in N-free sand culture in a naturally lit glasshouse from July to November in Perth, Western Australia.

Feeding of ^{14}C-Amino-Compounds. Application of ^{14}C-labeled compounds dissolved in xylem sap to cut transpiring shoots was as detailed elsewhere (15). Each shoot received 1 to 5 μCi of uniformly ^{14}C-labeled Arg, Asp, Gln, Asn, Ser, Glu, or Val over a period of 10 to 20 min. Shoots were then harvested immediately or transferred to unlabeled xylem sap for 10 to 220 min before harvest. Phloem sap was collected from petioles and fruits (11, 13), and the parent shoots then harvested for autoradiography or assay of ^{14}C in plant organs.

Autoradiography. Plant material was frozen in liquid N_2 and autoradiographed while still frozen or after freeze-drying (−30 C and 0.1–0.2 torr). Autoradiographs of whole shoots were also made on oven-dried material (80 C for 3 days).

Distribution of Insoluble ^{14}C in Tissues of Leaflets. Leaflet material was fixed in glutaraldehyde and embedded in glycol-methacrylate (2). Serial paradermal sections of 5-μm thickness were cut and assayed for ^{14}C by liquid scintillation spectrometry. Each sixth section of the series was examined microscopically to determine the proportions and areas of the different tissue types present.

Analysis of ^{14}C and Solutes of Phloem Sap. Phloem sucrose concentration was measured by refractometry. Estimations of amino compounds and assay of these for ^{14}C were as described elsewhere (6, 15).

Assay of ^{14}C in Plant Parts. Shoots were divided into leaflets, stem + petiole segments, vegetative apices of shoots (including stem and leaf tissue associated with leaves up to half fully expanded), and flowers and fruits (if present). These parts were oven-dried at 105 C and the dry material ground and assayed for ^{14}C using a Schöniger combustion technique (5). The CO_2 from combustion was collected in 2 methoxyethanol-ethanolamine (4:1, v/v) and assayed for ^{14}C by liquid scintillation spectrometry. Correction for quenching was made by external standardization.

RESULTS

Autoradiographic Localization of ^{14}C after Feeding ^{14}C-Amino-Compounds to Cut Shoots via Xylem (Fig. 1). Feeding shoots of various ages with ^{14}C-labeled Val, Arg, Asn, Gln, or Ser resulted in high densities of label in the vascular strands of stem and leaf and less intense labeling in the interveinal regions of leaflets (Fig. 1, B–H). This vein-dominated pattern of labeling was evident in autoradiographs of shoots harvested after a chase of from 0 to 220 min in unlabeled xylem sap after the 20-min pulse of label, indicating that ^{14}C did not reach interveinal areas of leaflets in any quantity (see Fig. 1, G and H).

The dicarboxylic amino acids ([^{14}C]Asp and [^{14}C]Glu) showed much less retention of ^{14}C in stems and heaviest labeling in regions of leaflets distant from the main veins (Fig. 1, A and I). This pattern was established within 30 min of applying the label and remained evident in shoots harvested after a 220-min chase in unlabeled sap. A feeding period with [^{14}C]Asp of only 10 or 15 min produced autoradiographs with veins labeled as densely or

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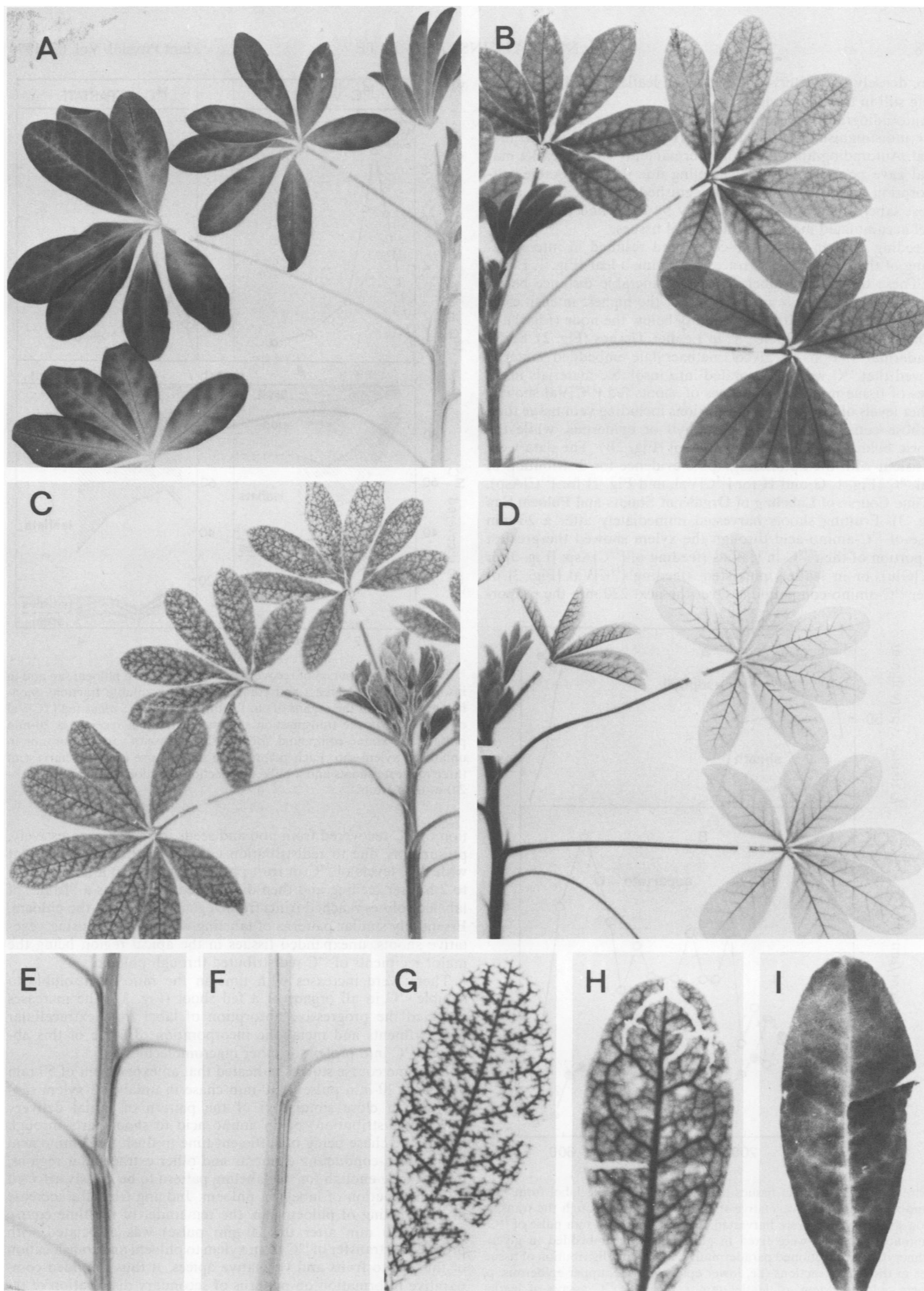

FIG. 1. Autoradiographs of upper regions of shoots (A–D), midregions of stems (E and F), and mature leaflets (G–I), from shoots of *L. albus* fed ^{14}C-amino-acid via the transpiration stream. ^{14}C-Amino-acids fed: [^{14}C]Asp (A, F, and I); [^{14}C]Asn (B); [^{14}C]Val (C, E, G, and H); [^{14}C]Arg (D). Experimental times: 20-min pulse of ^{14}C-amino-acid, 20-min chase in unlabeled xylem sap (A–F); 20-min pulse, 30-min chase (G and I); 20-min pulse, 180-min chase (H).

more densely than interveinal regions of leaflets, much of the ^{14}C being still in transit in xylem.

Autoradiographic patterns obtained using oven-dried material were indistinguishable from those derived from freeze-dried material. Autoradiographs of whole, permanently frozen leaflet material gave poor resolution of labeling due to considerable self-absorption of the ^{14}C, but still distinguished between amino acids whose label was absorbed principally by veins and those whose label accumulated mainly in interveinal tissues.

Feeding of each ^{14}C-amino-compound resulted in intense labeling of the three vascular traces supplying a leaf (Fig. 1, E and F). These traces were labeled at a considerable distance below their point of departure into a leaf, but the highest intensities of label were encountered at and slightly below the node (Fig. 1).

Distribution of Insoluble ^{14}C in Leaflet Tissues (Fig. 2). Serial paradermal sections of glycol methacrylate-embedded material showed that ^{14}C was incorporated into insoluble materials in all types of tissue in a leaflet. Leaves of shoots fed [^{14}C]Val showed higher levels of insoluble ^{14}C in sections including vein tissue than in those consisting of only mesophyll or epidermis, while the reverse held for labeling with ^{14}C[Asp] (Fig. 2B). The data were consistent with the autoradiographic evidence for distribution of total ^{14}C (Fig. 1, G and H for [^{14}C]Val, and Fig. 1I for [^{14}C]Asp).

Time Course of Labeling of Organs of Shoots and Phloem Sap (Fig. 3). Fruiting shoots harvested immediately after a 20-min pulse of ^{14}C-amino-acid through the xylem showed the greatest proportion of their ^{14}C in leaflets (feeding of [^{14}C]Asp; [Fig. 3] or [^{14}C]Glu), or in leaflets plus stem (feeding [^{14}C]Val [Fig. 3] or other ^{14}C-amino-compounds). Over the next 220 min the proportion of ^{14}C recovered from pod and seeds increased progressively, presumably due to redistribution of ^{14}C via phloem. Consistent with this, levels of ^{14}C in fruit phloem sap rose to a maximum 1 to 2 h after feeding and then diminished (Fig. 3), as a "front" of labeled solutes reached fruits from vegetative parts in the phloem. Essentially similar patterns of labeling were obtained using vegetative shoots, unexpanded tissues in the apical region being the major recipients of ^{14}C redistributed through phloem.

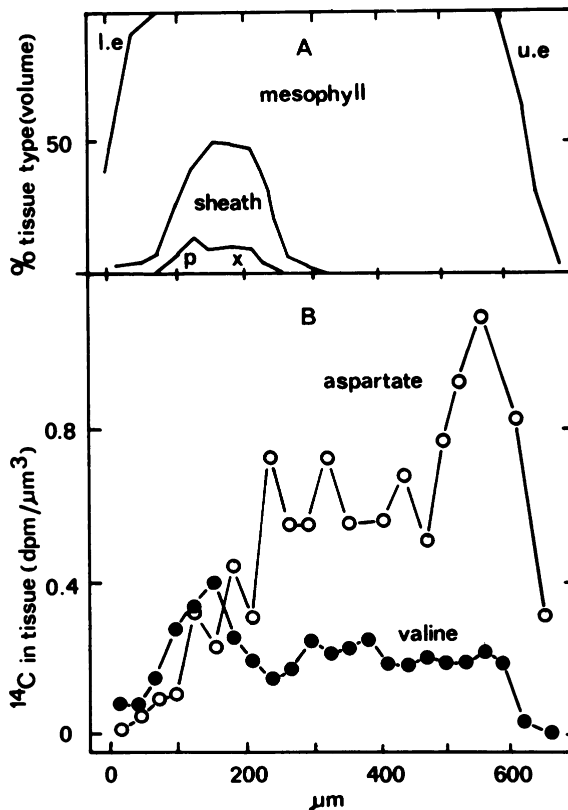

FIG. 2. Distribution in tissues of leaflets of insoluble label from ^{14}C-amino-acids fed to cut vegetative shoots of *L. albus* through the transpiration stream. Shoots were harvested 160 min after a 20-min pulse of ^{14}C-amino-acid. Leaflets were fixed in glutaraldehyde embedded in glycomethacrylate and sectioned paradermally. A: per cent distribution of tissue types in the leaflet sections (l.e, lower epidermis; u.e, upper epidermis; p, phloem, and x, xylem, of leaflet minor vein). B: ^{14}C assays of leaflet sections corresponding to distribution of tissues shown in A.

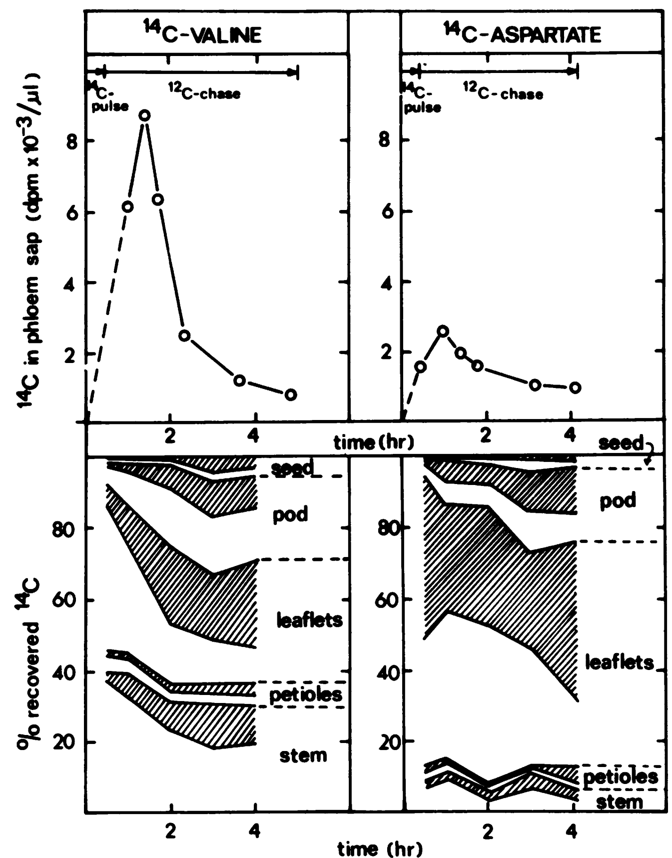

FIG. 3. Time courses of recovery of label in fruit tip phloem sap and in insoluble (hatched areas) and ethanol-extracted, soluble fractions (non-hatched areas) of the organs of cut fruiting shoots of *L. albus* fed [^{14}C]Val or [^{14}C]Asp via the transpiration stream. Each shoot received a 20-min pulse of ^{14}C-amino-compound, followed by a chase of varying duration in unlabeled xylem sap. Each point in the time course involved harvest of three replicate shoots and a bulked collection of phloem sap from all (18–24) of their fruits.

There were increases with time in the ratio of insoluble to soluble ^{14}C in all organs of a fed shoot (Fig. 3). The increases reflected the progressive absorption of label from extracellular compartments and metabolic incorporation of some of this absorbed ^{14}C into protein or other macromolecules.

The time course studies indicated that an experiment of 50 min duration (20-min pulse + 30-min chase in unlabeled xylem sap) provided a close simulation of the pattern of initial delivery (primary distribution) of an amino acid to shoot parts through xylem, the chase being of sufficient time to flush free amino acid from xylem-conducting elements and other extracellular regions, but not long enough for the labeling pattern to be grossly affected by redistribution of label via phloem. Judging from the increase in ^{14}C labeling of phloem sap, the remainder of the time course (*i.e.* 30–220 min after the 20-min pulse) was associated with progressive transfer of ^{14}C from xylem to phloem and translocation of this ^{14}C to fruits and vegetative apices. It thus provided comparative information on patterns of secondary distribution of the ^{14}C of the various amino compounds within the detached shoot.

Primary Distribution of ^{14}C-Amino-Acids to Shoots in Xylem (Figs. 4 and 5). Using the 50-min experimental period suggested above, primary distribution of label from various ^{14}C-amino-acids was studied in shoots of varying age (Fig. 4). The proportion of label abstracted by the shoot axis (stem + petioles) varied greatly between the amino compounds, amounting to 78% of the ^{14}C recovered in shoots fed [^{14}C]Arg, from 43 to 56% for shoots fed [^{14}C]Val, from 30 to 58% for [^{14}C]Asn and [^{14}C]Gln, but only 11 to 20% in the case of shoots fed [^{14}C]Asp. This distribution was consistent with the autoradiographic data (Fig. 1).

Inflorescences and fruits received only 1 to 5% of the ^{14}C during the 50-min experiment, a finding consistent with the low transpiration rates of these parts (12). In nonflowering shoots the apical region of the shoot (marked "vegetative apices" in Fig. 4) acquired from 9 to 20% of the label, suggesting a somewhat higher rate of transpirational loss from this region than in the case of reproductive organs.

The primary distribution of amino acids via the xylem to the leaves and stem segments up a shoot was likely to be determined by three factors. First, the particularly intense labeling of vascular tissue of departing leaf traces by amino acids (Fig. 1, E and F) suggested that a large proportion of the primary uptake by a segment of the shoot axis occurred without a lowering of the concentration of N solutes in the main xylem stream ascending to organs higher up the shoot. Upper leaves would thus transpire a xylem fluid whose concentration was not decreased to the extent expected from the proportional uptake of ^{14}C by the stem. Second, the highly active and selective absorption apparatus of the leaf traces was interpreted as carrying the potential for lowering significantly concentrations and proportions of N solutes moving to the leaves, and, if varying in intensity from node to node, the capacity for regulating differentially the N supply to individual leaves. Third, transpiration rates per unit leaf dry matter were found to be about two to five times higher in the uppermost expanded leaves than in those at the bottom of the shoot (McNeil, unpublished data). This gradient in transpirational activity would tend to offset any reduction in delivery to upper leaves caused by progressive uptake of ascending solutes by stem vascular tissue.

The interaction of these three effects was evident when the levels of ^{14}C in dry matter of leaflets and stem segments + petioles were studied for shoots harvested 30 min after a 20-min pulse of ^{14}C-amino-acid. Leaflets located high on the stem were as intensely labeled ([^{14}C]Asp or [^{14}C]Arg feeding) or more heavily labeled ([^{14}C]Val feeding) than those lower down the stem (Fig. 5). This effect was evident regardless of whether there had been weak ([^{14}C]Asp) or strong ([^{14}C]Arg and [^{14}C]Val) absorption by stem + petiole, or whether absorption by stem + petioles had been more concentrated in the upper ([^{14}C]Val) or the lower ([^{14}C]Arg) regions of the shoot (Fig. 5).

Secondary Distribution of ^{14}C of Xylem-fed ^{14}C-Amino-Compounds (Table I). This was studied, as suggested above, by measuring changes in distribution of ^{14}C among shoot parts in the period 30 to 220 min after administering a pulse of ^{14}C-amino-acid through the xylem (see time courses of labeling in Fig. 3). The amounts of ^{14}C incorporated into fruits during this period were equivalent to 15% of the ^{14}C fed to shoots as Asn, and 9.8,

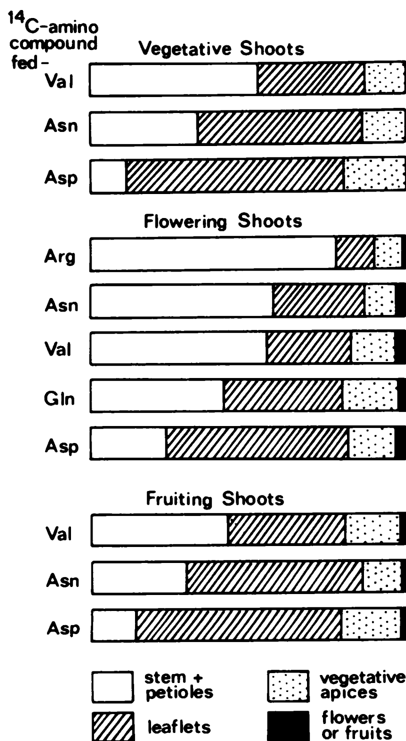

FIG. 4. Distribution of ^{14}C between organs of vegetative shoots, flowering shoots, and fruiting shoots of *L. albus* fed various ^{14}C-amino-compounds singly via the transpiration stream. Each study involved eight shoots fed a 20-min pulse of the ^{14}C-amino-acid, followed by a 30-min chase with unlabeled xylem sap. (Vegetative apices included stem and leaf tissue associated with leaves up to half fully expanded.)

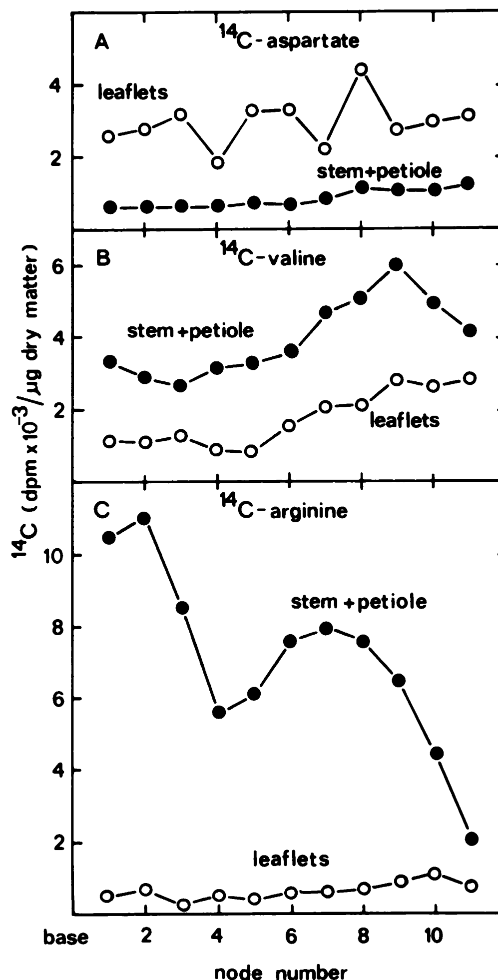

FIG. 5. Distribution of ^{14}C between the successive petiole + stem segments and leaflets of leaves of cut shoots of *L. albus* fed [^{14}C]Asp (A), [^{14}C]Val (B), or [^{14}C]Arg (C) via the transpiration stream. The shoot was fed a 20-min pulse (5 µCi/plant) of the labeled amino acid in xylem fluid followed by a 30-min chase in unlabeled xylem fluid. (Autoradiographs of upper portions of shoots of similar age fed with these ^{14}C-amino-acids are shown in Fig. 1.) Leaflets and stem + petiole segments are numbered from the base of the shoot upward according to node number.

TABLE I. Distribution of ^{14}C in Fruiting Shoots of White Lupin (*Lupinus albus* L. cv Ultra) fed ^{14}C-labeled Amino Compounds through the Transpiration Stream[1]

	fed compound	% of recovered ^{14}C still as fed compound	in other amino compounds	in non-amino compounds
(a) Fruit phloem sap[2]	^{14}C-Val	96	3	1
	^{14}C-Asn	82	7	11
	^{14}C-Asp	3	29	68
	^{14}C-Arg	0.5	28	71.5
(b) Ethanol-soluble fraction leaflets	^{14}C-Val	91	8	1
	^{14}C-Asn	76	7	17
	^{14}C-Asp	4	47	49
	^{14}C-Arg	21[3]		79

[1] Pulse of label fed for 20 min, shoots harvested after a 220 min 'chase' in unlabeled xylem sap.
[2] Average composition of sap collected from stalk and tip of fruits. Petiole phloem sap showed a virtually identical pattern of ^{14}C labeling to the phloem sap of fruits.
[3] Still as fed compound + in other amino compounds.

7.2, and 5.3% of the fed ^{14}C in the case of shoots receiving ^{14}C-labeled Val, Asp, and Arg, respectively.

The effectiveness in transfer of ^{14}C from a xylem amino compound to fruits was related to the extent to which it and derived labeled products passed from xylem to phloem. The distribution of ^{14}C among labeled compounds (Table I) showed that [^{14}C]Val and [^{14}C]Asn were readily transferred to phloem and were stored as such in the soluble phase of the leaflets. In contrast little ^{14}C from [^{14}C]Asp or [^{14}C]Arg was transferred to phloem and that which was transferred was largely associated with solutes other than the fed compounds. The significant labeling of non-amino compounds in leaflets following feeding of [^{14}C]Arg and [^{14}C]Asp suggested utilization of these compounds as N sources in the metabolism of these organs.

DISCUSSION

A recent study modeling the transport and utilization of recently assimilated C and N in nodulated white lupin (11) drew attention to the role of mature stem and leaves in abstraction of N from xylem and in the transfer by phloem of some of this N to centers of growth or storage in root or shoot. This paper examined these aspects of shoot transport in relation to the common nitrogenous solutes exported from nodulated roots to shoot in the xylem.

Great diversity was displayed in patterns of primary uptake from xylem. Some amino acids (*e.g.* Arg) were removed very effectively by vascular tissue of stem, petiole, and major veins of leaflets with little passage of the compound to interveinal regions of leaves; others (*e.g.* Val, Asn, Gln) were fairly equally shared among stem, petiole, and leaflets, whereas others (*e.g.* Asp and Glu) were retrieved only weakly by stems and accumulated largely in the nonvascular regions of leaflets. Equally variable was the accessibility of the amino acids to phloem and hence their capacity to nourish fruits and vegetative apices. Some (*e.g.* Val and Asn) were transferred to phloem largely in unmetabolized form; in others (*e.g.* Asp and Arg) metabolic products of the administered compound rather than the compound itself were loaded.

Ionic interactions of cell walls with xylem solutes, and membrane-based selectivity in cells lining the xylem pathway were probably major factors in determining the contrasting patterns of primary distribution of xylem amino acids. Solutes in predominantly cationic form (*e.g.* Arg) at a xylem pH of 5.5 to 6.5, were likely to have been subject to adsorption on negatively charged cell walls, anions (*e.g.* Asp and Glu) prevented from doing so by co-ion exclusion. For the predominantly neutral solutes (*e.g.* Asn, Gln, and Val) distribution was suggested to be determined largely by the capacity and selectivity of membrane-based uptake sites in cells lining the xylem, and similar mechanisms might have accounted for the high rates of uptake and metabolism of adsorbed Arg in stems and of Asp and Glu by cells of mesophyll.

Regardless of the amino acid fed, the departing vascular traces to leaves were active in uptake from xylem. At their point of departure at a node, where the traces appeared to absorb most strongly, xylem parenchyma transfer cells were abundant and showed extensive labyrinths of wall ingrowths (J. S. Pate, unpublished). The high density of ^{14}C labeling probably resulted from absorption or adsorption of xylem solute by these specialized cells. A similar role for transfer cells in nodes of other species has been suggested (3).

The different patterns of distribution of xylem amino acids in shoots of *L. albus* were interpreted as representing collectively a versatile system for allocation and utilization of the N arising from roots. The presence of compounds not readily transferred as such to phloem (*e.g.* Arg, Asp, and Glu) had the effect of ensuring that some of the nitrogenous products of the root were retained by mature organs of the shoot. Other compounds readily exchanged from xylem to phloem (*e.g.* Asn, Gln, and Val) were of obvious importance in loading the upward and downward streams of translocate with N, thus maintaining growth of meristematic regions and developing fruits. Since amides were the principal solutes engaged in xylem to phloem traffic of *Lupinus* (15), phloem-fed organs would benefit nutritionally from the additional N carried by the amide grouping (1, 6). Finally, the presence of a significant fraction of the xylem-borne N in forms (*e.g.* Asp and Glu) which largely escaped the stem uptake system ensured that leaf mesophyll would derive some N for growth and synthesis of proteins essential for photosynthesis, even under conditions in which N supply from the root was limiting.

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