Plant Physiol. (1985) 77, 418–428
0032-0889/85/77/0418/11/$01.00/0

Comparative Distribution and Metabolism of Xylem-Borne Amino Compounds and Sucrose in Shoots of *Populus deltoides*

Received for publication April 23, 1984 and in revised form October 22, 1984

THOMAS C. VOGELMANN, RICHARD E. DICKSON*, AND PHILIP R. LARSON
United States Department of Agriculture Forest Service, North Central Forest Experiment Station, Forestry Sciences Laboratory, Rhinelander, Wisconsin 54501

ABSTRACT

The transport and metabolism of xylem-borne amino compounds and sucrose were investigated in rapidly growing shoots of cottonwood (*Populus deltoides* Bartr. ex Marsh.). ^{14}C-labeled glutamine, threonine, alanine, glutamic acid, aspartic acid, and sucrose were applied to the base of severed stems for transport in xylem. Distribution and metabolism of the compounds were followed with autoradiography, microautoradiography, and radioassay. Three utilization patterns were observed: (a) little alanine and sucrose was transported to the laminae of either mature leaves or developing leaves. These compounds were taken up from xylem free-space and utilized in adjacent tissue; (b) threonine also did not move into mature leaves but was translocated to developing leaves or utilized in the stem; (c) glutamic acid and aspartic acid were transported directly into the laminae of mature leaves via the xylem. Relatively less ^{14}C was retained in stems compared to the other compounds.

Metabolism of the test compounds also differed considerably. ^{14}C from amino acids moved primarily into organic acids and protein. The ^{14}C from sucrose was widely distributed among the chemical fractions, with a high percentage found in structural carbohydrates. Clearly, cottonwood stems contain efficient uptake and transfer systems that differentiate among various compounds moving from root to shoot in xylem.

The transport of nitrogenous compounds from root to shoot in xylem and the transfer of these compounds from xylem to phloem in the shoot has been investigated with intensive studies on legumes using modern techniques for the efficient collection of xylem and phloem sap (20). Such studies have examined in detail the movement of nitrogen from root to shoot and the cycling of both nitrogen and carbon within the plant (12, 16, 18, 29). Similar intensive studies have not been conducted on nonleguminous plants or trees. Most studies on the transport of nitrogen in trees have examined either naturally occurring products in xylem sap (2, 6, 24), followed changes in nitrogen transport after different fertilizer treatments (10, 28), or studied stem extracts of nitrogen storage products (7, 15, 23).

The two amides, glutamine and asparagine, are major transport compounds in trees and many other woody plants (24). These amides, as well as some other amino acids, move readily in both xylem and phloem to developing tissues (16). However, initial distribution within the plant differs considerably among plant species. For example, from 30 to 40% of the glutamine fed to severed lupin stems passed through the stem xylem and into mature leaves (12). In contrast, glutamine fed to cottonwood was largely removed from the xylem in the stem (5). Several other amino acids commonly found in xylem sap also differ widely in transport characteristics. For example, aspartic acid fed to the xylem of severed lupin stems accumulated primarily in the leaf mesophyll while arginine accumulated in stem and leaf vascular tissue (12). Metabolism of xylem-borne amino acids can also differ both during and after transport. Sharkey and Pate (26) found that asparagine was largely transferred unchanged from xylem to phloem while aspartic acid was metabolized to several other products before loading into the phloem. Our objective was to follow the transport, uptake, and metabolism of sucrose and the major amino acids found in cottonwood xylem sap after application of these ^{14}C-labeled compounds to the bases of severed stems.

MATERIALS AND METHODS

Plant Material. Cottonwood plants (*Populus deltoides* Bartr. ex Marsh.) were grown from seed in a controlled environment room under conditions previously described (5). However, in the present series of experiments, the plants had eight mature leaves (LPIs[1] 7–14) attached during treatment compared to three or seven mature leaves per plant in previous experiments. (5).

Application of Radioactive Compounds. Early in the treatment day, plants were removed from the growth room and preconditioned as before (5). After 1 h, the leaf at LPI 15 was removed and the plants were severed under water at the base of the internode subtending LPI 15. The severed plants were transferred to 50-ml beakers containing artificial xylem sap (5 mol m^{-3} KCl, 0.4 mol m^{-3} malic acid, adjusted to pH 5.4 with KOH) and placed in the treatment chamber. The plants were next transferred to vials containing 0.9 MBq of either [U-^{14}C]glutamine, -glutamic acid, -aspartic acid, -alanine, -threonine, or -sucrose per ml of artificial xylem sap. Each plant was fed 1 ml of radioactive solution (approximately 15 min) and then transferred back to unlabeled solution for the remainder of the translocation period. A single plant was treated for either 0.5, 1, or 3 h (feeding time plus chase with unlabeled solution) to provide a translocation-metabolic time series for each compound. The time series were not replicated because previous work (5) showed that uptake and distribution patterns were consistent from plant to plant, the extraction procedures were reliable (3), and distribution of ^{14}C in different tissues and chemical fractions across the time series provided a check on large errors.

Plant Harvest, Autoradiography, and ^{14}C Analyses. At the end of the translocation period, internode, petiole, and leaf segments were excised, frozen in liquid N_2, freeze-dried, pressure infiltrated with Spurr's low viscosity resin, sectioned, and prepared for microautoradiography (32). The remaining leaves and stem segments were pressed, quick-frozen in dry ice-liquid N_2 vapor, freeze-dried, and autoradiographed with Kodak Industrex X-ray

[1] Abbreviations: LPI, leaf plastochron index; GAB, gamma-aminobutyric acid.

film.

After freeze-drying and autoradiography, samples of lamina and stem (5–15 mg) were extracted with methanol:chloroform:water (12:5:3, v:v:v) and separated into seven chemical fractions with a combination of solvent, ion-exchange, and enzymic techniques (3). The seven fractions were $CHCl_3$ (lipids and pigments soluble in chloroform), protein (pronase extract of the pellet), starch (Clarase and Diazyme, Miles Laboratories[2], extract of the pellet), residue (tissue remaining after all extracts), sugar (neutral fraction from the ion exchange columns), organic acids (6 M HCOOH eluate from the anion column), and amino acids (4 M NH_4OH eluate from the cation column). Total recovery of ^{14}C (sum of the seven fractions) averaged about 95% based on NCS analysis of the tissue. The individual amino acids and organic acids were separated with TLC, scraped from the thin-layer plates into scintillation vials, and solubilized in NCS for ^{14}C determinations.

RESULTS

Localization of ^{14}C in Whole Leaves and Stem Internodes. The movement of radioactive amino acids into leaves after xylem-feeding cottonwood plants resulted in three general distribution patterns. (a) Alanine did not accumulate in mature leaves or in young leaves (Fig. 1A; Table I). (b) Threonine also did not accumulate in mature leaves but rapidly accumulated throughout the lamina in young developing leaves (Fig. 1B). (c) ^{14}C from glutamic and aspartic acid rapidly accumulated in the mesophyll of mature leaves (Fig. 1C).

The accumulation patterns of aspartic acid or glutamic acid indicated both xylem and phloem transport. In mature leaves, some loading was apparent in the major laterals and midvein after 1 h (Fig. 1C); however, most of the ^{14}C remained in the interveinal mesophyll. In developing leaves, initial accumulation of glutamic or aspartic acid (after 0.5 h) was primarily in lamina tips and marginal glands indicating xylem transport. Labeling of leaf glands was most noticeable in aspartic acid-treated plants (LPIs 0–2, Fig. 1C). With longer treatment time (3 h), ^{14}C concentration increased in the smaller veins and mesophyll.

The distribution of ^{14}C in the leaves after xylem-feeding sucrose differed from that obtained with amino acids. The major veins of all mature leaves were rapidly labeled (Fig. 1D); the ^{14}C from sucrose was confined to the veins and did not move into the interveinal mesophyll even after 3 h. Initially (after 0.5 h), young developing leaves contained little ^{14}C, and that present was confined to the vascular tissue. At 3 h, vein labeling had increased and some ^{14}C had accumulated in the interveinal mesophyll.

Specific activity of ^{14}C in the laminae quantitatively confirmed the autoradiographic patterns (compare Fig. 1 with Table I). For example, the concentration of ^{14}C in leaves at LPI 8 reflected the

[2] Mention of trade names is for the convenience of the reader and does not constitute endorsement by the United States Department of Agriculture Forest Service.

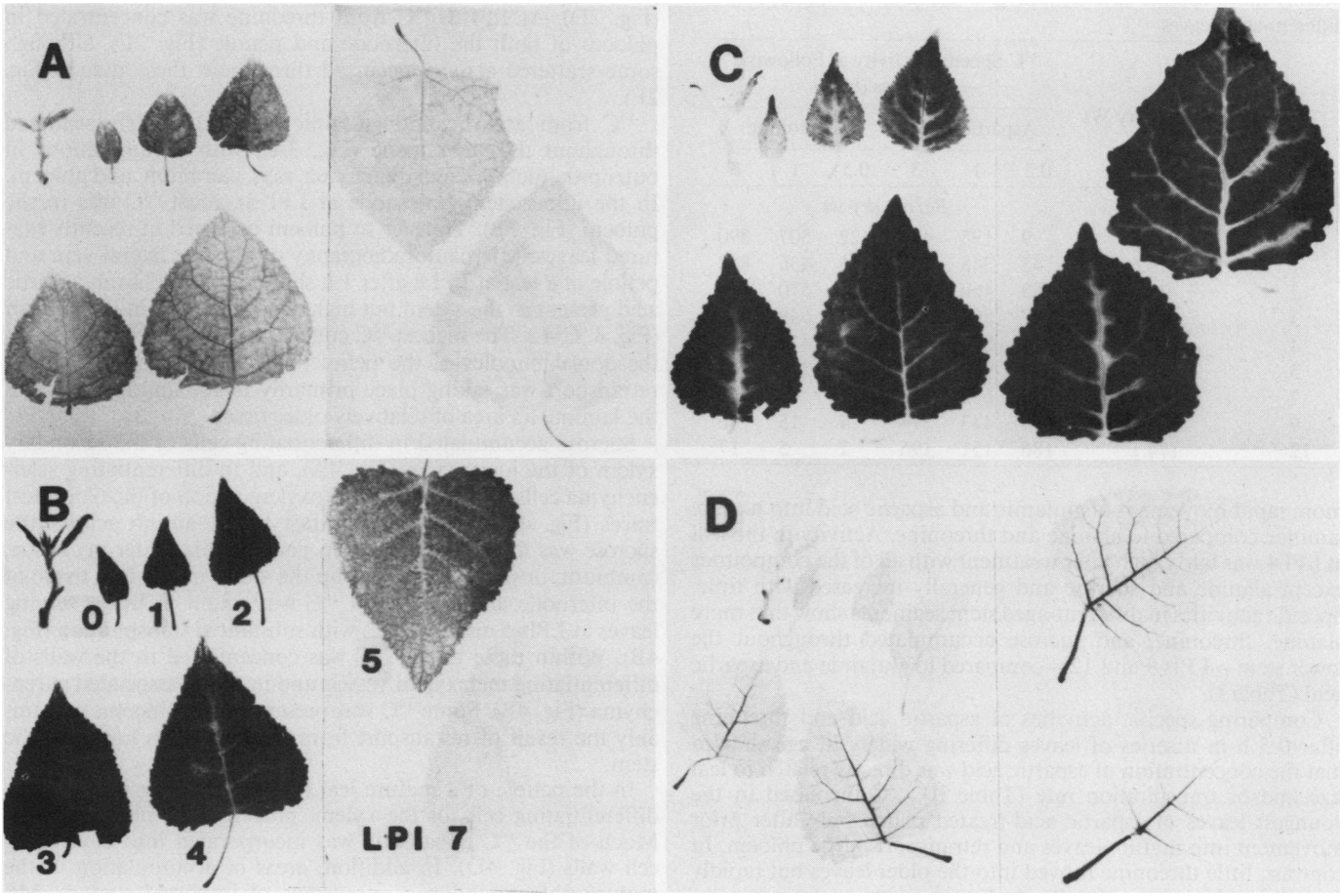

FIG. 1. Autoradiographs of whole cottonwood leaves after xylem-feeding different amino acids and sucrose. The plants were treated for 15 min (0.9 MBq ^{14}C), harvested 1 h after the ^{14}C-labeled compounds were applied, freeze-dried, pressed, and autoradiographed. A, Alanine-; B, threonine-; C, aspartic acid-; and D, sucrose-treated plants. Each series shows a recently matured leaf and five or six developing leaves plus the terminal bud and elongating internodes. See threonine (B) for the LPI numbering sequence. The lamina of LPI O was 2 cm long.

Table I. *Specific Activity of ^{14}C in Laminae and Stems of Cottonwood after Xylem-Feeding Radioactive Amino Acids and Sucrose*

Plants were fed 0.9 MBq ^{14}C-amino acids or sucrose through the xylem of the severed stem and then transferred to a nonradioactive solution for the indicated translocation times. Specific activity (Bq/mg dry wt plant material) is the sum of ^{14}C recovered in each of seven chemical fractions separated during chemical analysis of the different plant tissue. Stem segments LPI 4, 9, and 12 consisted of the node and internode directly subtending the leaf of that age and contained both xylem and phloem.

	^{14}C Specific Activity at Following Times (h)														
	Laminae						Stems								
	LPI 4			LPI 8			LPI 4			LPI 9			LPI 12		
	0.5	1	3	0.5	1	3	0.5	1	3	0.5	1	3	0.5	1	3
	Bq/mg dry wt														
Glutamic acid	274	412	504	75	228	108	272	900	1504	813	2005	516	921	1539	1275
Aspartic acid	200	602	573	543	306	328	262	295	319	462	742	287	346	922	438
Alanine	71	88	256	13	33	52	884	992	589	797	1799	1036	1768	3711	2113
Threonine	103	481	480	6	14	21	723	801	760	2090	1622	1295	2862	1757	2364
Sucrose	66	68	255	94	163	79	311	454	1666	1226	1108	2342	1118	1161	1861

Table II. *Specific Activity of ^{14}C in Developing Leaves of Cottonwood after Xylem-Feeding ^{14}C-Labeled Aspartate and Threonine*

Specific activity is expressed as Bq/mg dry wt of lamina tissue. Leaves were freeze-dried, ground, subsampled, and solubilized in NCS to determine the ^{14}C concentration. Lamina dry weights are averages obtained from 16 plants grown under the same experimental conditions. Dry weights times specific activity will give the total ^{14}C present in the different-aged leaves.

LPI	Lamina Dry Wt	^{14}C Specific Activity at Following Times (h)					
		Aspartic acid			Threonine		
		0.5	1	3	0.5	1	3
	mg	*Bq/mg dry wt*					
0	9.4	9	195	454	122	507	890
1	16.6	45	348	487	135	436	868
2	31.5	65	460	506	216	570	848
3	54.5	197	802	696	189	649	786
4	86.5	315	854	795	131	413	456
5	125.5	389	988	850	40	211	212
7	164.8	506	632	607	7	15	14
9	196.7	664	443	574	4	15	6
12	174.1	186	151	196	2	5	17

more rapid movement of glutamic and aspartic acid into mature laminae compared to alanine and threonine. Activity in the leaf at LPI 4 was fairly high after treatment with all of the compounds except alanine and sucrose and generally increased with time. Specific activities in different-aged stem segments show that more alanine, threonine, and sucrose accumulated throughout the lower stem—LPIs 9 and 12—compared to glutamic and aspartic acid (Table I).

Comparing specific activities of aspartic acid and threonine after 0.5 h in a series of leaves differing widely in age showed that the concentration of aspartic acid was directly related to leaf size and/or transpiration rate (Table II). ^{14}C increased in the youngest leaves of aspartic acid-treated plants only after prior movement into mature leaves and retransport in the phloem. In contrast, little threonine moved into the older leaves but rapidly accumulated in young developing leaves.

Microautoradiography of Stem and Leaf Tissues. Microautoradiography showed that alanine and threonine uptake by the stems was similar. ^{14}C rapidly accumulated in the xylem parenchyma, rays, and differentiating cells of the cambial zone in the lower stem (Fig. 2, A and B). Concentrations were particularly high in the metaxylem parenchyma of traces serving mature leaves on the lower stem, in ray cells, and in vessel-associated parenchyma cells (Fig. 2C). Little ^{14}C accumulated in phloem of the lower stem. However, in the upper stem (internode LPI 7), considerable [^{14}C]threonine had accumulated in the phloem—primarily in traces serving recently matured and expanding leaves (Fig. 2D). At LPI 3, ^{14}C from threonine was concentrated in phloem of both the internode and petiole (Fig. 2E), although some scattered activity occurred throughout these tissues (Fig. 2F).

^{14}C from aspartic and glutamic acid was widely scattered throughout the stem tissue (Fig. 3A), with accumulations in outer pith, metaxylem parenchyma, rays, cambium, and phloem. In the upper stem (internode at LPI 3), most ^{14}C was in the phloem (Fig. 3B). Transfer to phloem occurred in recently matured leaves. Microautoradiography of a major lateral vein and petiole of a leaf at LPI 8 after 1 h showed little ^{14}C from aspartic acid present in the xylem but high concentrations in the phloem (Fig. 3, C–E). The highest ^{14}C concentrations were in phloem of the dorsal bundles of the petiole, indicating that loading and retransport was taking place primarily in the apical portion of the lamina, an area of relatively older tissue.

Sucrose accumulated in differentiating cells of the secondary xylem of the lower stem (Fig. 4A), and in differentiating sclerenchyma cells located in the protoxylem region of the oldest leaf traces (Fig. 4A, arrow). In contrast to the amino acids, little sucrose was found in metaxylem parenchyma, older ray tissue, cambium, or phloem. Farther up the stem, in primary tissue of the internode at LPI 5, most ^{14}C was found in traces serving leaves at LPIs 5 and 4, leaves with substantial transpiration (Fig. 4B). Within these traces, ^{14}C was concentrated in the walls of differentiating metaxylem vessels and in vessel-associated parenchyma (Fig. 4B). Some ^{14}C was present in the phloem, presumably the result of retransport from mature leaves lower on the stem.

In the petiole of a mature leaf (Fig. 4C), ^{14}C accumulated in differentiating cells of the xylem, phloem, and phloem fibers. Much of the ^{14}C apparently was incorporated into developing cell walls (Fig. 4D). In addition, areas of accumulation in the mature phloem indicate retransport of imported sucrose. Microautoradiographs of a petiole at LPI 6 (Fig. 4, E and F) showed accumulation of ^{14}C in xylem parenchyma and in vessel cell walls. Little accumulated in phloem fibers because secondary wall development had not yet begun in the LPI 6 leaf.

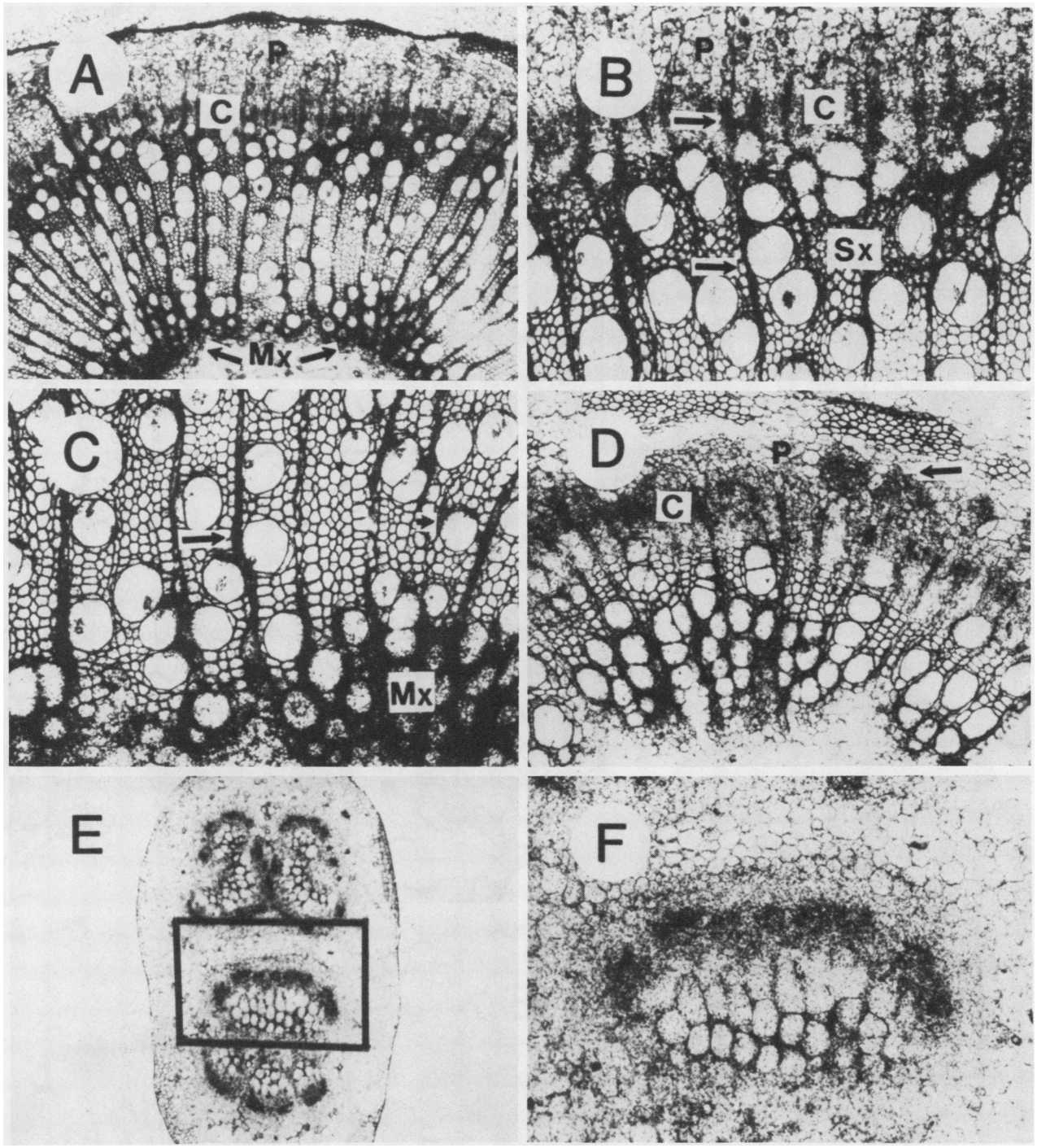

FIG. 2. Microautoradiographs of cottonwood internode and leaf transections after xylem-feeding labeled alanine or threonine. A–C, treatment: [U-^{14}C]alanine, 0.5 h (15-min pulse, 15-min chase); exposure, 8 d. A, Internode at LPI 12 showing short-term accumulation in metaxylem parenchyma (Mx), rays, developing secondary xylem, and cambium (× 65). B, Section from the upper left quadrant of A showing accumulation of ^{14}C in cambium (C), young developing rays (upper arrow), mature rays (lower arrow), and developing secondary xylem (Sx). Little ^{14}C accumulated in the phloem (P) (× 163). C, Section from lower right quadrant of A showing heavy accumulation of ^{14}C in metaxylem parenchyma, in ray parenchyma (arrow), and in vessel-associated parenchyma (dart) (× 163). D–F, treatment: [U^{14}C]threonine, 1 h (15-min pulse, 45-min chase); exposure, 8 d (D) and 40 d (E). D, Internode at LPI 7 showing high levels of ^{14}C in cambium, rays, and metaxylem parenchyma in old leaf trace (left) and phloem loading in an adjacent younger trace (right, arrow) (× 130). E, Mid-petiole of a leaf at LPI 3 showing ^{14}C concentrated in phloem and cell walls of developing xylem (× 58). F, Enlargement of boxed area of G. Note distribution of [^{14}C]threonine throughout the petiolar tissue (× 163).

Distribution of ^{14}C in Different Chemical Fractions of Leaves and Stems. In laminae at both LPIs 4 and 8, ^{14}C from the labeled amino acids moved rapidly into the organic acid fraction. For example, up to 40% of the ^{14}C was found in the organic acids within 0.5 to 1 h when aspartic acid was applied (Table III). The amino acids were also rapidly incorporated into protein, particularly in the developing leaf (LPI 4). ^{14}C incorporated into protein in LPI 4 ranged from 27% (aspartic acid) to 70% (threonine). Alanine differed from the other amino acids in the small amount of ^{14}C converted to organic acids and the large amount recovered

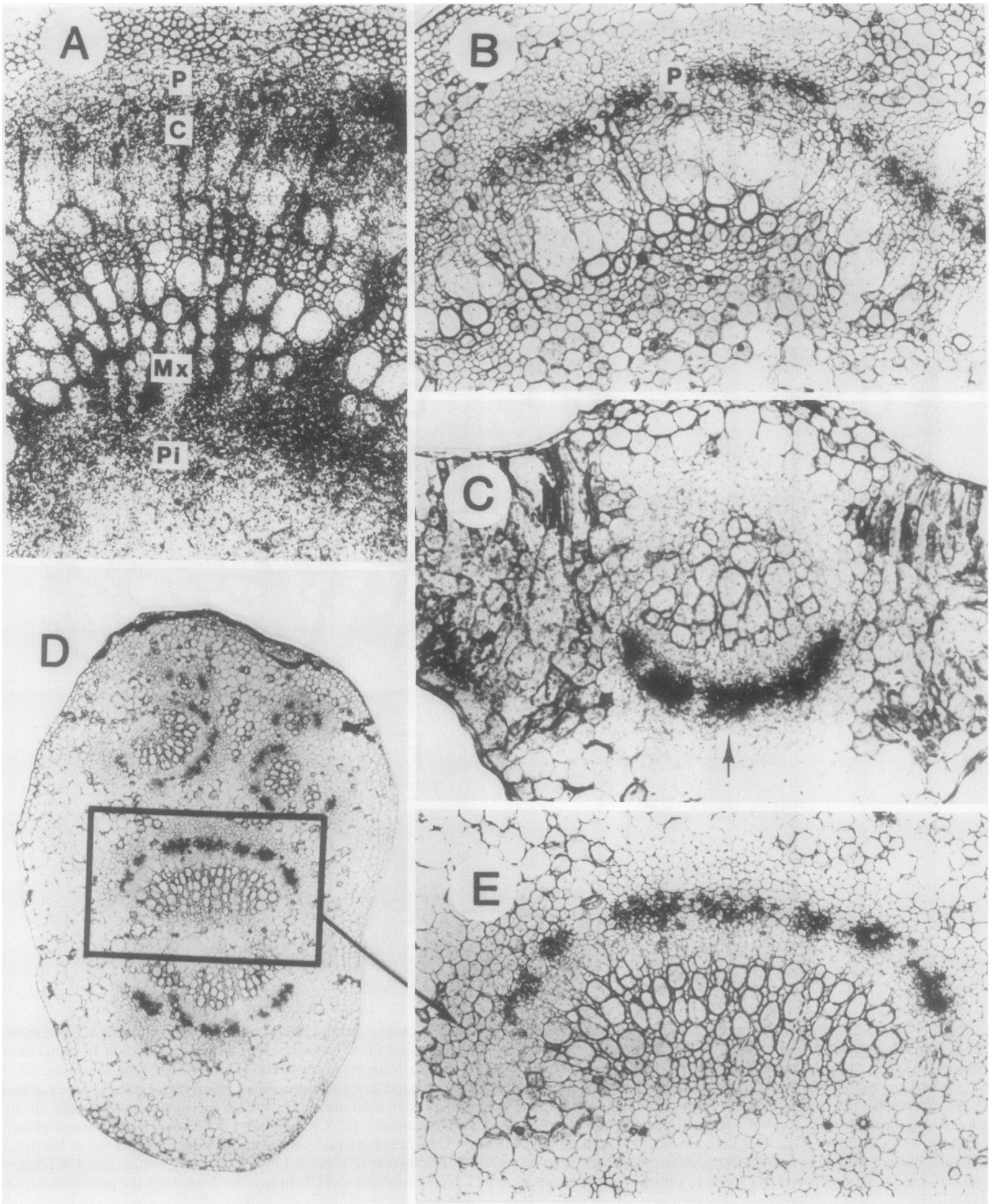

FIG. 3. Microautoradiographs of cottonwood internode and leaf transections after xylem-feeding labeled glutamic acid and aspartic acid. A and B, treatment: [U-^{14}C]glutamic acid, 1 h (15-min pulse, 45-min chase); exposure, 10 d. A, Internode at LPI 8 showing ^{14}C scattered throughout the stem with some accumulation in outer pith (Pi), metaxylem parenchyma (Mx), rays, and cambium (C) with less in the phloem (P) (× 130). B, Internode at LPI 3 showing ^{14}C located mainly in the phloem (P) (× 163). C and D, Treatment: [U-^{14}C]aspartic acid, 1 h (15-min pulse, 45-min chase); exposure, 40 d. C, a Major lateral vein of a mature leaf at LPI 8 showing ^{14}C concentrated in the adaxial phloem (arrow); other phloem bundles were unlabeled (× 205). D, Mid-petiole of a mature leaf at LPI 8 showing accumulation of ^{14}C in phloem. Although ^{14}C was present in phloem of all vascular traces, the highest concentrations occurred in the dorsal bundles (boxed area and below) (× 52). E, Enlargement of the boxed area of D showing ^{14}C concentrated in the phloem but little in the xylem and other tissues (× 130).

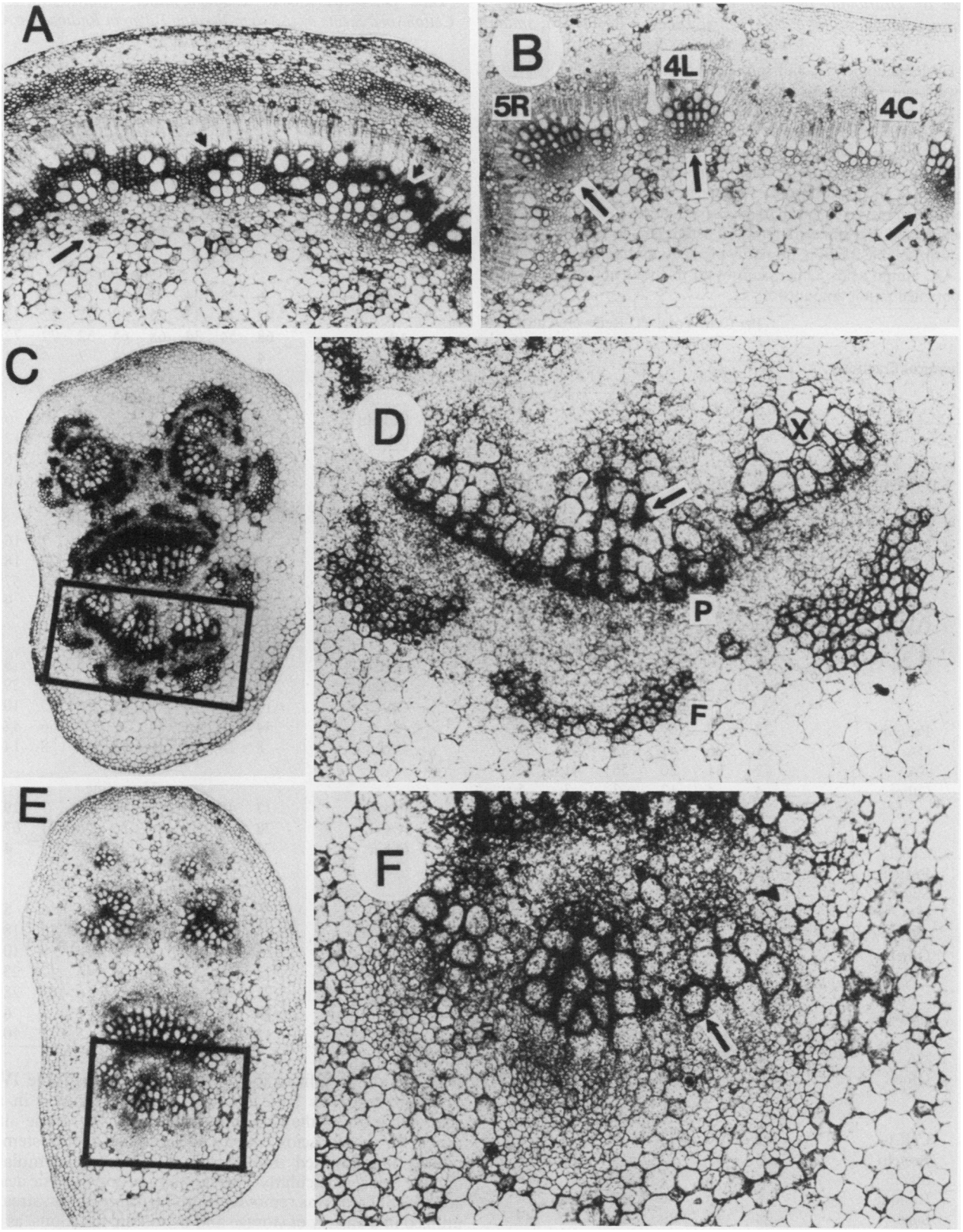

FIG. 4. Microautoradiographs of cottonwood internode and leaf transections after xylem-feeding labeled sucrose. A–F, treatment: [U-^{14}C]sucrose, 1 h (15-min pulse, 45-min chase); exposure, 5 d (C and E), 10 d (A), and 20 d (B). A, Internode at LPI 7 showing ^{14}C from sucrose concentrated in the differentiating xylem (darts). At high magnification, much of the ^{14}C appeared to be associated with or incorporated in the xylem cell walls. Differentiating sclerenchyma cells in the region of obliterated protoxylem of older leaf traces also accumulated ^{14}C (arrow) (× 65). B, Internode at LPI 5 showing ^{14}C concentrated in metaxylem (arrows) of traces serving leaves at LPIs 5 and 4; *e.g.* right trace of LPI 5 (5R) and left (4L) and central (4C) traces of LPI 4 (× 65). C, Mid-petiole of a leaf at LPI 9 showing ^{14}C concentrated in differentiating xylem and phloem fibers (× 52). D, Enlargement of boxed area of C. Xylem (X), phloem fibers (F), and vessel-associated parenchyma cells (arrow). Note weak ^{14}C loading of phloem (P) (× 150). E, Mid-petiole of a leaf at LPI 6 with ^{14}C located mostly in the xylem (× 52) F, Enlargement of boxed area of E. Differentiating metaxylem vessels (arrow) and vessel-associated parenchyma are most heavily labeled (× 150).

Table III. *Distribution of ^{14}C among Major Chemical Fractions in Cottonwood Laminae after Xylem-Feeding Different Radioactive Amino Acids and Sucrose*

^{14}C-labeled amino acids and sucrose were fed through the xylem of the lower stem for 15 min and then chased with unlabeled solution. Plants were harvested at the times shown, freeze-dried, and subsampled (5–15 mg). The subsample was then separated into seven chemical fractions. One plant was used for each treatment time and each amino acid. LPI 4 was a developing leaf about one-half expanded. LPI 8 was a fully expanded and exporting leaf. Percentages are based on the total ^{14}C recovered in the seven chemical fractions (see sucrose for these fractions). The petiole and midvein were removed before lamina analyses. $CHCl_3$, chloroform-soluble fraction, primarily pigments and lipids; OA, organic acids; AA, amino acids; Other, sum of all fractions not actually listed that contained minor amounts of ^{14}C.

Chemical Fractions	Per Cent Total ^{14}C Recovered at Following Times (h)					
	LPI 4			LPI 8		
	0.5	1	3	0.5	1	3
	%					
Glutamine						
Protein	18	19	41	6	6	20
OA	32	28	27	33	32	35
AA	40	42	18	44	48	33
Other	10	11	14	18	14	12
Glutamic acid						
Protein	11	16	29	6	8	20
OA	20	22	16	16	22	18
AA	62	55	40	74	65	52
Other	7	7	15	4	5	10
Aspartic acid						
Protein	23	29	27	8	14	19
OA	31	36	41	33	42	51
AA	42	31	20	56	41	25
Other	4	4	12	3	3	5
Alanine						
$CHCl_3$	18	9	16	6	12	12
Protein	38	36	41	12	11	15
Sugar	4	4	5	10	18	20
OA	13	18	18	19	17	20
AA	24	29	15	50	39	30
Other	3	4	5	3	3	3
Threonine						
Protein	35	68	70	12	35	45
OA	2	2	2	39	12	8
AA	58	22	13	26	31	15
Other	5	8	15	23	22	32
Sucrose						
$CHCl_3$	9	6	13	4	3	6
Protein	25	19	27	13	10	14
Starch	6	5	5	2	1	4
Residue	6	6	6	3	2	7
Sugar	34	40	29	58	57	49
OA	11	16	14	12	21	17
AA	9	8	6	8	6	3

in the $CHCl_3$ fraction. ^{14}C from sucrose was also rapidly incorporated into protein and was widely distributed throughout the other chemical fractions in developing laminae.

The distribution of ^{14}C among the chemical fractions in stem segments differed from that found in laminae (Table IV). For example, relatively large amounts of ^{14}C remained in amino acids for most of the experimental period. Threonine and alanine, two amino acids heavily accumulated in the stem, were rapidly incorporated into protein. ^{14}C again accumulated in organic acids, particularly when glutamine and aspartic acid were applied. Sucrose was removed from the transpiration stream and utilized in a variety of storage and metabolic functions, as shown by the wide distribution of ^{14}C among the different chemical fractions. Particularly important was the utilization in structural development (residue fraction) in the older stem segments.

Table IV. *Distribution of ^{14}C among Major Chemical Fractions in Cottonwood Stems after Xylem-Feeding Different Radioactive Amino Acids and Sucrose*

Plants treated as in Table III. The stem segments given below comprised the node and internode subtending leaves at LPIs 4, 9, and 12 and contained both xylem and phloem.

Chemical Fractions	Per Cent Total ^{14}C Recovered at Following Times (h)								
	LPI 4			LPI 9			LPI 12		
	0.5	1	3	0.5	1	3	0.5	1	3
	%								
Glutamine									
Protein	6	8	24	2	4	14	2	3	8
OA	22	34	35	25	28	27	22	22	26
AA	68	54	34	68	64	52	72	72	53
Other	4	4	7	5	4	7	4	3	13
Glutamic acid									
Protein	6	7	11	5	7	21	10	6	16
OA	22	15	17	14	13	13	15	12	10
AA	68	74	64	77	74	55	67	76	65
Other	4	4	8	4	6	11	8	6	9
Aspartic acid									
Protein	11	11	14	7	14	19	7	16	19
OA	30	40	33	28	10	24	20	18	18
AA	52	44	47	61	70	48	68	57	50
Other	7	5	6	4	6	9	5	9	13
Alanine									
$CHCl_3$	8	4	13	4	3	7	3	3	5
Protein	25	35	40	24	31	33	18	29	27
OA	15	27	14	8	19	16	5	10	10
AA	44	20	18	54	32	27	66	44	43
Other	8	14	15	10	15	17	8	14	15
Threonine									
Protein	17	48	65	13	40	66	7	29	53
AA	78	46	25	84	55	26	91	67	42
Other	5	6	10	3	5	8	2	4	5
Sucrose									
$CHCl_3$	6	6	4	4	5	6	6	5	4
Protein	23	24	20	16	17	19	16	18	22
Starch	12	14	13	8	9	16	9	10	8
Residue	12	16	31	18	19	46	17	23	24
Sugar	21	19	13	35	36	6	33	28	14
OA	14	14	14	7	6	2	8	6	6
AA	12	7	5	12	8	5	11	10	22

Metabolic Changes within the Amino Acid and Organic Acid Fractions. Xylem-fed glutamine was rapidly deaminated in both leaves and stems and the resulting glutamic acid partially converted to GAB (Table V). Glutamic and aspartic acids were

Table V. *Distribution of ^{14}C among Individual Amino Acids from Cottonwood Laminae and Stems after Xylem-Feeding Different Radioactive Amino Acids and Sucrose*

The amino acids were separated from other water-alcohol-soluble compounds with ion exchange and then isolated with TLC. Amino acid standards were spotted with the experimental solution on the TLC plates. After separation and visualization, the standard spots with the unknown ^{14}C were scraped into scintillation vials for counting. Data shown are from the 1-h treatment. Percentage changes over time (0.5–3 h) show that ^{14}C decreased in the fed amino acid and increased in other amino acids. Threonine was not determined in Leaf LPI 8 because there was not enough ^{14}C in the AA fraction for reliable counts after separation. All amino acids analyzed are listed under sucrose.

Amino Acids	Per Cent Total Amino Acids				
	Laminae		Stems		
	LPI 4	LPI 8	LPI 4	LPI 9	LPI 12
			%		
			Glutamine		
Glu	45	44	58	50	41
Asp	2	3	7	8	3
Gln	1	2	6	20	19
GAB	50	47	20	17	32
Other	2	4	9	5	5
			Glutamic acid		
Glu	53	47	69	42	34
Asp	2	2	9	7	6
Gln	1	1	5	11	19
GAB	42	49	13	37	33
Other	2	1	4	3	8
			Aspartic acid		
Glu	2	2	9	13	16
Asp	96	93	78	68	58
GAB	0	1	8	13	18
Other	2	4	5	6	8
			Alanine		
Glu	46	19	44	38	24
Asp	3	0	27	23	10
Ala	24	67	9	10	25
GAB	21	8	11	21	32
Other	6	6	9	8	9
			Threonine		
Thr	80	—	94	94	95
Other	20	—	6	6	5
			Sucrose		
Glu	21	18	34	32	18
Asp	7	19	6	12	5
Asn	4	7	1	1	1
Gln	15	10	2	2	3
Ser	22	22	23	20	16
Thr	8	5	3	2	2
Ala	17	12	10	17	35
GAB	6	7	21	14	20

slowly metabolized to other amino acids in both leaves and stems. Glutamic acid was primarily converted to GAB in leaves, and to aspartic acid and glutamine in stems. Up to 30% of the tracer fed as [^{14}C]glutamic acid was recovered as [^{14}C]glutamine after 3 h in the stem at LPI 12. Glutamic acid and glutamine are readily interconverted in cottonwood stems although glutamic acid production is favored. Little aspartic acid was converted to other amino acids in leaves. More than 90% of the ^{14}C was still in aspartic acid after 3 h. In stems, however, aspartic acid was converted to glutamic acid and GAB. After 3 h (data not shown), only 30% of the ^{14}C was still in aspartic acid, while glutamic acid and GAB contained 20% and 36%, respectively. Alanine was rapidly converted to glutamic acid and aspartic acid in both young leaf and stem tissues. In contrast to other amino acids, little threonine was converted to either organic acids or to the other amino acids analyzed (Tables III–V).

The small amount of ^{14}C from sucrose that was incorporated into the amino acid fraction of young leaves and stems (Tables III and IV) was found in several amino acids (Table V). The high percentage of ^{14}C in serine is interesting because none of the amino acids contributed significant ^{14}C to serine in any tissue or at any treatment time.

The percentage distribution of ^{14}C among the organic acids often differed between leaves and stems after treatment with a particular amino acid (Table VI). For example, malic acid was lightly labeled in laminae but heavily labeled in stems after glutamine treatment. In both leaves and stems, malic acid and succinic acid contained high levels of ^{14}C after aspartic acid, alanine, and glutamic acid treatment. Citric acid (from alanine, aspartic acid, and sucrose) and 2-ketoglutaric acid (from glutamic acid and alanine) also contained considerable ^{14}C. Phosphorylated compounds (origin of the TLC plate) were heavily labeled after certain treatments (glutamine and alanine, LPI 8; alanine and aspartic acid, stems; sucrose, both leaves and stems).

DISCUSSION

The nitrogen requirements of different plant parts vary widely, depending on the developmental stage of the particular organ and of the whole plant. Young developing leaves or flowers have low transpiration rates and therefore must receive most of their nitrogen through the phloem (16, 29). Nitrogen in the phloem of developing leaves originates in mature leaves and from xylem to phloem transfer in the stem (4, 18).

Uptake from the Xylem and Transfer to the Phloem. Initial uptake from the xylem free-space takes place in the metaxylem, vessel-associated parenchyma, and ray parenchyma. These tissues form the major stem storage pools for both long-term storage in the fall (14, 25) and short-term storage involved in primary xylem to secondary xylem, xylen-to-phloem, and phloem-to-xylem transfer. Such storage tissues buffer short-term and diurnal (17) fluctuations of nitrogen in both xylem and phloem.

In developing cottonwood shoots without secondary tissue, the metaxylem parenchyma cells are active in uptake and storage of both amino acids and sugars (Figs. 2–4). In the lower stem of cottonwood and in other plants with considerable secondary development, the ray parenchyma are the major uptake and storage tissues (5, 8). Sauter (25) has proposed that initial uptake from the vessels takes place in 'contact cells,' specialized ray cells active in loading and unloading the vessels. After uptake from the vessels, movement in rays results in both primary xylem-to-secondary xylem and xylem-to-phloem transfer. Autoradiographs of leaves and microautoradiographs of both leaf and stem transections from plants fed different amino acids or sucrose via the transpiration stream illustrate that each of the fed compounds has a somewhat different uptake (movement from the xylem free-space into the symplast) and distribution pattern. Such patterns are the product of the physical environment of the apoplast and the membrane transfer characteristics of the symplast.

Numerous studies have shown that xylem sap is slightly acidic (usual pH range of 5.0–6.0) and is maintained in this acidic state largely through the metabolism of malic acid and other strong organic acids (1, 9, 21). The pH of cottonwood xylem sap (5.3–5.6) is near the optimum found for alanine and glutamine uptake

Table VI. *Distribution of ^{14}C among Individual Organic Acids from Cottonwood Laminae and Stems after Xylem-Feeding Different Radioactive Amino Acids and Sucrose*

The organic acids were separated from other water-alcohol-soluble compounds with ion exchange and then isolated with TLC. The different spots (corresponding to the location of standards run on the same TLC sheet) were scraped from the TLC sheets and counted with liquid scintillation spectrometry. Data shown are from the 1-h treatment. The origin contains sugar phosphates and other phosphorylated compounds that did not move with this solvent system. Threonine was not included because there was not enough ^{14}C in the organic acids to count.

Organic Acids	Per Cent Total Organic Acids				
	Laminae		Stems		
	LPI 4	LPI 8	LPI 4	LPI 9	LPI 12
			%		
			Glutamine		
Origin	19	51	11	8	7
Citrate	6	9	16	6	8
Malate	15	10	32	54	68
2-Ketoglutarate	6	4	10	12	4
Succinate	49	20	24	18	12
Fumarate	5	6	7	2	1
			Glutamic acid		
Origin	11	12	5	5	5
Citrate	5	5	6	4	6
Malate	18	18	45	48	68
2-Ketoglutarate	17	22	7	27	8
Succinate	34	39	31	13	12
Fumarate	15	4	6	3	1
			Aspartic acid		
Origin	7	5	29	17	8
Citrate	10	6	19	8	18
Malate	63	74	26	39	36
2-Ketoglutarate	7	3	8	7	18
Succinate	12	10	16	24	18
Fumarate	1	2	2	5	2
			Alanine		
Origin	5	21	36	20	16
Citrate	14	13	13	14	8
Malate	49	29	22	36	19
2-Ketoglutarate	6	14	5	4	27
Succinate	11	14	22	24	26
Fumarate	15	9	2	2	4
			Sucrose		
Origin	40	49	23	23	24
Citrate	14	18	22	22	28
Malate	21	21	25	26	18
2-Ketoglutarate	6	5	10	9	10
Succinate	16	6	17	18	17
Fumarate	3	1	3	2	3

by tomato stems (30). The uptake of amino acids by cottonwood stems follows the sequence alanine > threonine > glutamic acid > aspartic acid (lower stem, Table I). This is the same general uptake pattern of basic > neutral > acidic amino acids found in tomato stems (30). The ionization state of the amino compound is probably not as important in uptake as membrane configuration, membrane affinity for a particular amino acid, and pH effect on trans-membrane carriers (11).

Sucrose uptake from the xylem free-space by the xylem parenchyma was so efficient in the lower stem, petioles, and major leaf veins that little of the applied sucrose reached the mesophyll of either mature or developing leaves. The rapid sucrose utilization by the vascular tissue of cottonwood and the restricted transfer to phloem was probably related to the sucrose concentration applied and contrasts with other xylem-feeding studies with sucrose. Sacalis and Durkin (22) applied [^{14}C]sucrose in a 2% sucrose carrier to cut roses and found considerable ^{14}C-labeled sucrose was transferred from xylem to phloem and moved up the stem to the flowers. In our work with cottonwood, no additional unlabeled sucrose was added to the artificial xylem sap. If the concentration of sucrose were increased (lower specific activity), more [^{14}C]sucrose probably would be transferred from xylem to phloem in mature leaves for retransport in the phloem to developing leaves. This concentration effect may also be an important factor in the uptake and distribution patterns obtained with the amino acids. For example, alanine was heavily concen-

trated in the xylem parenchyma of the stem (Fig. 2C) with little movement into either mature or developing leaves. Van Bel *et al.* (31) found that low concentrations of alanine increased uptake rate in tomato stems. They suggested that alanine was concentrated and retained in parenchyma cells near the vessels at low input concentrations. At higher input concentrations, movement away from the supplying vessels would increase but uptake rate would decrease and more alanine would move through the system. Similar results were found for glutamine in cottonwood (Dickson, unpublished). All of the amino acids and sucrose in our studies were supplied at low concentrations and high specific activities, which would favor maximum uptake and retention by xylem parenchyma.

Three distribution patterns based on movement into leaves and uptake by stems were observed in cottonwood. (a) Alanine and sucrose were rapidly taken up and retained in the stem and major leaf traces with little movement to either phloem or developing leaves. Threonine and glutamine (5) were rapidly removed from the xylem free-space in the stem and transferred from primary xylem to secondary xylem, from xylem to phloem, then translocated in the phloem to developing leaves. (c) Aspartic and glutamic acid were less readily taken up in the stem and moved into the mature leaves. The movement of aspartic and glutamic acid into mature leaves provided a ready source of amino nitrogen for protein synthesis and maintenance in these leaves. In addition, phloem loading and retransport from these leaves would normally be directed to the lower stem and roots. Thus, aspartic and glutamic acid may be important in recycling amino nitrogen from shoots to roots in cottonwood.

Metabolic Conversion during Uptake and Distribution. Conversion to organic acids or incorporation into protein were the two major metabolic pathways followed by these xylem-applied amino acids (Tables III and IV). The rapid incorporation of threonine and alanine into protein is consistent with their accumulation in areas of developing tissue of both stems and leaves (Fig. 2). In addition, these amino acids were probably utilized in protein synthesis by mature parenchyma cells. Similar incorporation into protein of mature tissue was found in *Pisum* after xylem-feeding several different amino compounds (19). ^{14}C from sucrose rapidly appeared in most of the major chemical fractions, indicating that its utilization was different from that of the amino acids. The large amount of ^{14}C in the residue supports the microautoradiographic evidence for incorporation into developing cell walls (Fig. 4).

The distribution of ^{14}C within the amino acid and organic acid fractions extracted from different plant tissues showed that conversion of one amino acid to another was limited and largely confined to compounds closely related to the fed compound (Table V). Glutamine was rapidly deaminated in both leaves and stems and the resulting glutamic acid converted to GAB. This was unexpected because the transport amides are resistant to metabolism (26). However, a similar conversion was reported for glutamine in pea (29).

Threonine metabolism was particularly interesting because it was incorporated primarily into protein with little apparent metabolism to other amino acids (Tables III–V). However, analysis of the individual amino acids of protein extracted from young developing cottonwood leaves (pronase extract, 6 N HCl hydrolysis, TLC separation) showed that more than 50% of the ^{14}C from [^{14}C]threonine was in isoleucine (Dickson, unpublished). Threonine contained most of the remainder of the ^{14}C, although small amounts of ^{14}C (up to 9%) were also found in valine. Threonine is readily converted to isoleucine (13). However, we did not analyze for isoleucine in the soluble amino acid fractions because it is not a major constituent of xylem sap (Table V).

All of the organic acids separated by TLC contained some ^{14}C, which indicates the rapid movement of ^{14}C from these xylem-fed compounds into the tricarboxylic acid cycle (13). Malic acid usually contained the most ^{14}C but succinic acid, citric acid and 2-ketoglutaric acid also contained high levels. A similar distribution of ^{14}C in organic acids was found in apple after feeding [^{14}C]glutamic acid and [^{14}C]aspartic acid to roots (27). Pyruvic acid, an important intermediate of alanine metabolism, was lost during extraction or TLC separation of organic acids. This may be part of the reason for the low content of ^{14}C in the organic acid fraction after alanine treatment.

The rapid metabolism of these xylem-borne compounds in cottonwood was consistent with other xylem- (12, 26, 29) or root-feeding (27) studies. Although certain pathways predominate, the transport amino acids—glutamine, asparagine, glutamic acid, and aspartic acid—are major intermediates in nitrogen metabolism and provide direct access to the tricarboxylic acid cycle (13). Thus, the carbon and nitrogen supplied by these compounds can be transported throughout the plant and converted to almost any final product, depending on the current demands of the plant.

LITERATURE CITED

1. Butz RG, RC Long 1979 L-Malate as an essential component of the xylem fluid of corn seedling roots. Plant Physiol 64: 684–689
2. Dickson RE 1979 Xylem translocation of amino acids from roots to shoots in cottonwood plants. Can J For Res 9: 374–378
3. Dickson RE 1979 Analytical procedures for the sequential extraction of ^{14}C-labeled constituents from leaves, bark and wood of cottonwood plants. Physiol Plant 45: 480–488
4. Dickson RE, PR Larson 1981 ^{14}C fixation, metabolic labeling patterns, and translocation profiles during leaf development in *Populus deltoides*. Planta 152: 461–470
5. Dickson RE, TC Vogelmann, PR Larson 1985 Glutamine transfer from xylem to phloem and translocation to developing leaves of *Populus deltoides*. Plant Physiol 77: 412–417
6. Ferguson IB, NA Turner 1981 Mobilization of macro-nutrients in cuttings of kiwifruit (*Actinida chinensis* Planch.). Ann Bot 47: 229–237
7. Hill-Cottingham PG, CP Lloyd-Jones 1973 Seasonal variations in absorption and metabolism of carbon-14-labeled arginine in intact apple stem tissue. Physiol Plant 29: 35–44
8. Höll W 1975 Radial transport in rays. *In* MH Zimmermann, J A Milburn, eds, Encyclopedia of Plant Physiology, New Series, Vol 1, Transport in Plants, I, Phloem Transport. Springer-Verlag, Berlin, pp 432–450
9. Israel DW, WA Jackson 1982 Ion balance, uptake, and transport processes in N_2-fixing and nitrate- and urea-dependent soybean plants. Plant Physiol 69: 171–178
10. Kato T 1981 Major nitrogen compounds transported in xylem vessels from roots to top in citrus trees. Physiol Plant 52: 275–279
11. Kinraide TB, B Etherton 1980 Electrical evidence for different mechanisms of uptake for basic, neutral, and acidic amino acids in oat coleoptiles. Plant Physiol 65: 1085–1089
12. 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
13. Miflin BJ, PJ Lea 1977 Amino acid metabolism. Annu Rev Plant Physiol 28: 299–329
14. Nelson EA, RE Dickson 1981 Accumulation of food reserves in cottonwood stems during dormancy induction. Can J For Res 11: 145–154
15. O'Kennedy BT, MJ Hennerty, JS Titus 1975 Changes in the nitrogen reserves of apple shoots during the dormant season. J Hortic Sci 50: 321–329
16. Pate JS 1980 Transport and partitioning of nitrogenous solutes. Annu Rev Plant Physiol 31: 313–340
17. Pate JS, CA Atkins, K Hamel, DL McNeil, DB Layzell 1979 Transport of organic solutes in phloem and xylem of a nodulated legume. Plant Physiol 63: 1082–1088
18. Pate JS, CA Atkins, DF Herridge, DB Layzell 1981 Synthesis, storage and utilization of amino compounds in white lupin (*Lupinus albus* L.) Plant Physiol 67: 37–42
19. Pate JS, TP O'Brien 1968 Microautoradiographic study of the incorporation of labelled amino acids into insoluble compounds of the shoot of a higher plant. Planta 78: 60–71
20. Pate JS, PJ Sharkey, OAM Lewis 1975 Xylem to phloem transfer of solutes in fruiting shoots of legumes, studied by a phloem bleeding technique. Planta 122: 11–26
21. Raven JA, FA Smith 1976 Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol 76: 415–431

22. SACALIS JN, D DURKIN 1972 Movement of ^{14}C in cut roses and carnations after uptake of ^{14}C-sucrose. J Am Soc Hortic Sci 97: 481–484
23. SAGISAKA S 1974 Effect of low temperature on amino acid metabolism in wintering poplar. Arginine-glutamine relationships. Plant Physiol 53: 319–322
24. SAUTER JJ 1981 Seasonal variation of amino acids and amides in the xylem sap of *Salix*. Z Pflanzenphysiol 101: 399–411
25. SAUTER JJ 1982 Efflux and reabsorption of sugars in the xylem. I. Seasonal changes in sucrose efflux in *Salix*. Z Pflanzenphysiol 106: 325–336
26. SHARKEY PJ, JS PATE 1975 Selectivity in xylem to phloem transfer of amino acids in fruiting shoots of white lupin (*Lupinus albus* L.). Planta 127: 251–262
27. SPENCER P, JS TITUS 1971 Translocation of glutamate-^{14}C and aspartate-^{14}C by intact apple trees. J Am Soc Hortic Sci 96: 131–133
28. TROMP J, JC OVAA 1979 Uptake and distribution of nitrogen in young apple trees after application of nitrate or ammonium, with special reference to asparagine and arginine. Physiol Plant 45: 23–28
29. URQUHART AA, KW JOY 1982 Transport, metabolism, and redistribution of xylem-borne amino acids in developing pea shoots. Plant Physiol 69: 1226–1232
30. VAN BEL AJE, P VAN LEEUWENKAMP, C VAN DER SCHOOT 1981 Amino acid uptake by various tissues of the tomato plant. Effects of external pH and light. Z Pflanzenphysiol 104: 117–128
31. VAN BEL AJE, E MOSTERT, AC BORSTLAP 1979 Kinetics of L-alanine escape from xylem vessels. Plant Physiol 63: 244–247
32. VOGELMANN TC, RE DICKSON 1982 Microautoradiography of water-soluble compounds in plant tissue after freeze-drying and pressure infiltration with epoxy resin. Plant Physiol 70: 606–609