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Glutamine Transfer from Xylem to Phloem and Translocation to Developing Leaves of *Populus deltoides*

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

ABSTRACT

The distribution of ^{14}C from xylem-borne [^{14}C]glutamine, the major nitrogen compound moving in xylem sap of cottonwood (*Populus deltoides* Bartr. ex Marsh), was followed in rapidly growing shoots with a combination of autoradiographic, microautoradiographic, and radioassay techniques. Autoradiography and ^{14}C analyses of tissues showed that xylem-borne glutamine did not move with the transpiration stream into mature leaves. Instead, most of it was transferred from xylem to phloem in the upper stem and then translocated to young developing tissues. Microautoradiography showed that metaxylem parenchyma, secondary xylem parenchyma, and rays were the major areas of uptake from xylem vessels in the stem. Accumulation in phloem (high ^{14}C concentrations in sieve tubes) took place in internodes subtending recently mature leaves. Little ^{14}C from xylem-borne glutamine was found in phloem of mature leaves, which indicates restricted retransport of glutamine that did enter the leaf. In the primary tissues of the upper stem, most ^{14}C was found in the phloem. Cottonwood stems have an efficient uptake and transfer system that enhances glutamine movement to developing tissues of the upper stem.

In many species, the root system is the major source of organic nitrogen used by the developing shoot. Nitrogen metabolism in roots can be very specific, resulting in the synthesis and transport of only one or two major nitrogen compounds (11). In cottonwood (*Populus deltoides* Bartr. ex Marsh.), glutamine is the major organic nitrogen compound translocated from roots to shoots in the xylem sap, although several other amino compounds are also present (2). Intensive chemical studies on legume xylem and phloem sap have shown longitudinal xylem transport, radial xylem to xylem transfer, and radial xylem to phloem transfer, all of which direct nutrients toward the sink tissue (8, 9, 12, 15). Studies of similar intensity have not been reported for nonleguminous species, although xylem to phloem transfer of urea (13) and amino acids (14) has been demonstrated in apple trees. This paper reports a study in which [^{14}C]glutamine was applied to the distal end of exposed xylem of severed cottonwood shoots. Xylem movement, xylem to phloem transfer, and general distribution of ^{14}C from xylem-fed glutamine in shoots were then analyzed with whole leaf autoradiography, scintillation counts of tissues, and an improved method of microautoradiography that localizes water-soluble nutrients (16).

MATERIALS AND METHODS

Plant Material. Cottonwood plants (*Populus deltoides* Bartr. ex Marsh.) were grown from seed in a controlled environment as previously described (2). Photon flux density during the 18-h day was 300 μmol s^{-1} m^{-2} measured with a Li-Cor[1] qantum sensor (400–700 nm) and meter (models LI-1095 and LI-185A, respectively). Day and night temperatures were 27 and 20°C, and the corresponding RH were 50 and 80% measured with a Belfort hygrothermograph calibrated with a Hg thermometer (Belfort Instrument Co., Baltimore, MD). The plants were treated at plastochron indices from 16 to 18 (7). At plastochron index 16, the 16th leaf from the base (the index leaf) was about 2 cm long and was assigned a LPI[2] of 0. Older leaves were numbered positively and consecutively down the plant. LPI 7 was the first fully expanded, mature leaf. Plastochron index 16 plants were approximately 20 to 25 cm tall.

[^{14}C]Glutamine Application. Three series of plants were treated with [^{14}C]glutamine—two series for determining uptake and distribution of xylem-fed glutamine and one series for microautoradiography. In the first distribution series, plants had three mature leaves, LPIs 7 to 9; leaves at LPI 10 and older were removed before treatment. Three replications were treated continuously with 0.9 MBq [U-^{14}C]glutamine (1.48 GBq mol^{-1} m^{-3}; radiochemical purity, 98%; Amersham) per ml of solution for 5, 15, 25, and 30 min. In the second distribution series, plants had seven mature leaves, LPIs 7 to 13; leaves at LPIs 14 and older were removed before treatment. Two replications were treated continuously with 0.18 MBq [U-^{14}C]glutamine for 5, 15, and 30 min. Plants in the first and second distribution series took up about 1 and 2 ml of solution in 30 min, respectively. Plants in the microautoradiographic series were treated for 0.5, 1, and 3 h (either continuously or pulse-chase) with 0.9 MBq [U-^{14}C]glutamine. Photon flux density during treatment was 200 μmol s^{-1} m^{-2} (GE fluorescent tubes F30 T12 CW RS). Temperature in the treatment chamber ranged from 25 to 28°C.

For treatment, plants were removed from the growth chamber, preconditioned for 1 h under low light intensity (4–8 μmol s^{-1} m^{-2}), and then severed at the root collar under tap water. Plants in the first distribution series were treated by immersing the stem base in a treatment solution containing [^{14}C]glutamine in xylem sap (root exudate diluted 1:10 [v:v] with distilled H_2O) and those in the second distribution series and microautoradiographic series were treated with [^{14}C]glutamine in artificial xylem sap (5 mol m^{-3} KCl, 0.4 mol m^{-3} malic acid adjusted to pH 5.4 with KOH). No differences either in plant response or glutamine distribution could be attributed to the different solutions.

Plant Harvest, Analysis of ^{14}C, and Autoradiography. Shoots were separated into segments (node plus the subtending internode); divided into xylem and phloem plus cortex; oven-dried

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

[2] Abbreviation: LPI, leaf plastochron index.

(70°C); ground to pass a 40-mesh Wiley screen; solubilized (24 h at 45°C) with NCS (Amersham); and assayed for ^{14}C by liquid scintillation spectrometry (LS-150, Beckman Instruments). After solubilization, the solutions were acidified with glacial acetic acid to overcome chemiluminescence—quenching was automatically corrected by the scintillation counter. Leaves were either oven-dried for ^{14}C analysis or freeze-dried for autoradiography before ^{14}C analysis.

Selected node, internode, petiole, and leaf segments from the microautoradiographic series were excised with a razor blade, frozen in liquid N_2, freeze-dried (−50°C, 7.0 Pa), and pressure infiltrated with low viscosity epoxy resin (16).

RESULTS

[^{14}C]Glutamine Distribution within the Plant. Within 5 min after placing the severed stem in the glutamine solution, ^{14}C was beginning to accumulate in the xylem subtending the mature leaves, LPIs 8 and 9 (Fig. 1A). As treatment time increased, ^{14}C slowly increased in the lower stem but rapidly accumulated in the xylem segments subtending the most recently matured (fully expanded) leaves (LPIs 7–9). The accumulation patterns were similar in the phloem plus cortex tissue that had been stripped from the xylem cylinder, but less ^{14}C was present than in the associated xylem segments (Fig. 1B). These accumulation patterns were essentially the same when based on either specific activity or total ^{14}C in the tissue (Fig. 1, A and B).

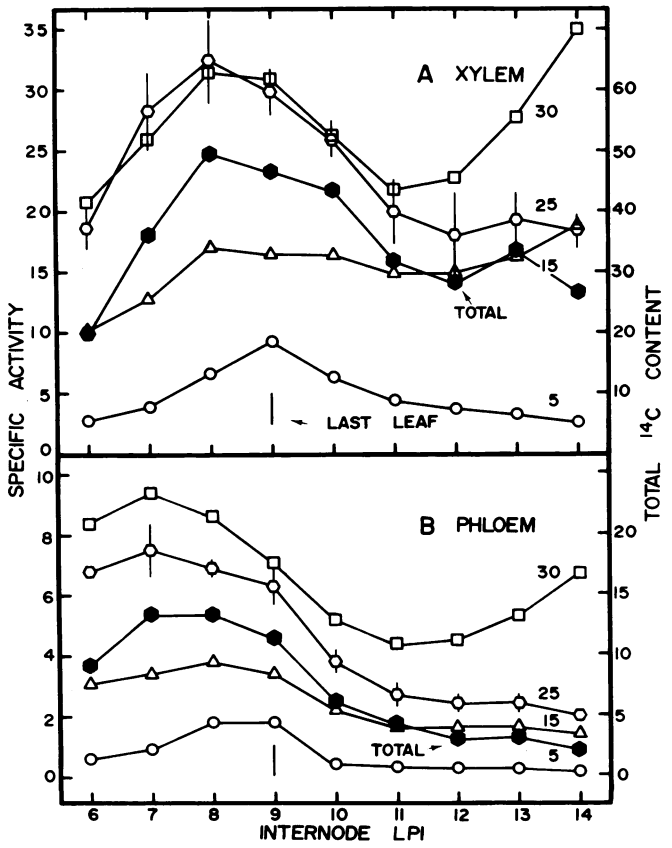

FIG. 1. ^{14}C in cottonwood stem segments after xylem feeding [U-^{14}C] glutamine—all mature leaves below LPI 9 removed. Note that LPIs decrease upward in the stem. A, Xylem; B, phloem plus cortex stripped from the xylem cylinder. Specific activity is expressed as Bq/mg dry wt × 10^{-2}, total ^{14}C as Bq × 10^{-3}. Stem segments contained both the node and internode subtending the leaf indicated by LPI. Treatment was a continuous application of 0.9 MBq [^{14}C]glutamine per ml in 1:10 xylem sap for 5 (○), 15 (△), 25 ( ), and 30 (□) min. Bars on the 25-min treatment indicate SE for three replications per treatment. Total ^{14}C content was from the 25-min treatment.

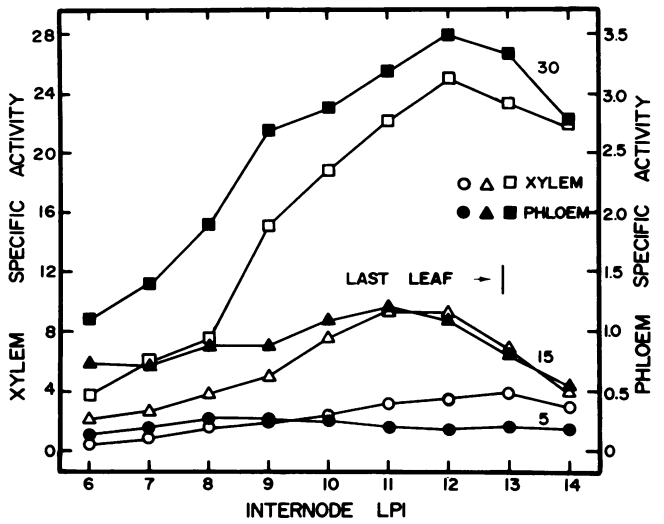

FIG. 2. ^{14}C in cottonwood stem segments after xylem-feeding [U-^{14}C] glutamine—all mature leaves below LPI 13 removed. (●, ▲, ■), Phloem-cortex; (○, △, □), xylem. Specific activity is expressed as Bq/mg dry wt × 10^{-2}. Treatment as in Figure 1, but with 0.18 MBq [^{14}C]glutamine per ml in artificial xylem sap for 5 (○, ●), 15 (△, ▲), and 30 (□, ■) min and with two replications per treatment.

Table I. *Total ^{14}C Accumulated in Different Aged Leaf and Stem Tissue after Xylem-Feeding [^{14}C]Glutamine*

Plants with seven mature leaves were treated continually with 0.18 MBq [^{14}C]glutamine in artificial xylem sap for the times given, freeze-dried, and analyzed for ^{14}C. Leaf tissue was the whole lamina with midvein and petiole removed. Xylem tissue was the xylem cylinder (node-internode) subtending the leaf at that LPI. Phloem-cortex was the tissue stripped from the xylem cylinder. It was not possible to separate xylem and phloem in primary tissue above LPI 6. Values are total Bq × 10^{-2} and are the average of two replications.

Tissue	Total ^{14}C Accumulated at Following Treatment Times (min)		
	5	15	30
	Bq × 10^{-2}		
Leaf (LPI)			
2	0.2	5.2	15.6
4	4.5	26.4	34.0
8	6.3	12.6	16.2
12	2.6	4.0	7.6
Xylem (LPI)			
6	4.0	19.1	35.3
8	19.1	42.7	77.7
10	37.7	111.4	186.7
12	66.8	161.2	270.7
14	52.2	64.7	272.1
Phloem-cortex (LPI)			
6	2.3	11.2	18.7
8	5.2	15.1	26.4
10	5.1	19.8	38.9
12	4.0	20.7	44.5
14	3.3	7.9	34.1

The pattern of accumulation shown in Figure 1, A and B, occurred in stems from which the older mature leaves (LPIs 10–

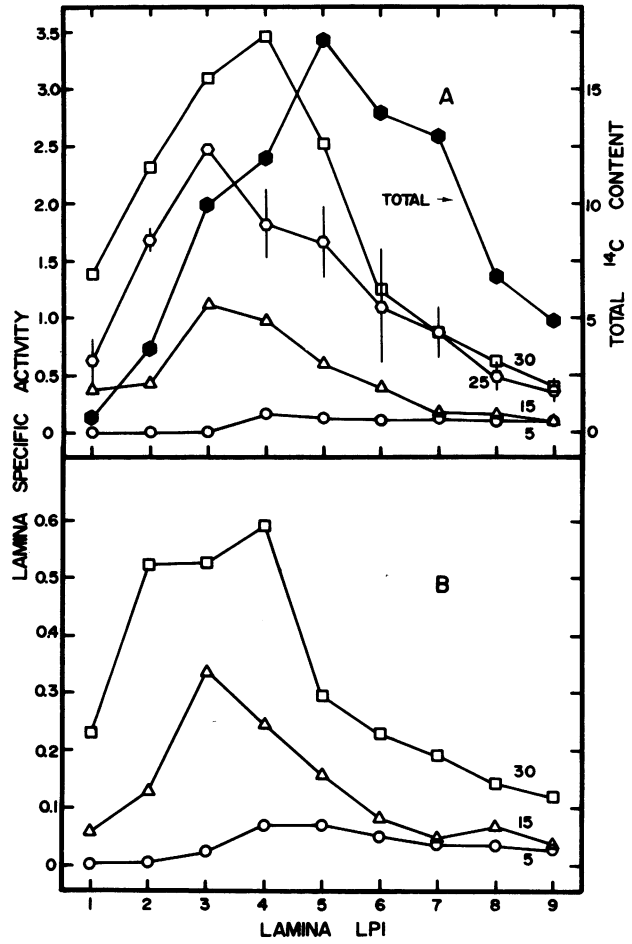

FIG. 3. ^{14}C in cottonwood laminae after xylem feeding [U-^{14}C]glutamine. A, Plants with mature leaves below LPI 9 removed and treated continuously with 0.9 MBq [^{14}C]glutamine. B, Plants with mature leaves below LPI 13 removed and treated continuously with 0.18 MBq [^{14}C]-glutamine. Specific activity expressed as Bq/mg dry wt $\times 10^{-2}$, total ^{14}C as Bq $\times 10^{-3}$. Plants in A and B took up about 0.9 MBq and 0.4 MBq in 30 min, respectively. Bars on the 25-min treatment (A) indicate SE for three replications. Total ^{14}C content was from the 25-min treatment.

14) had been removed before treatment with [^{14}C]glutamine. In plants with mature leaves down to LPI 13, ^{14}C accumulated in both xylem and phloem plus cortex in all internode segments associated with mature leaves (Fig. 2). Again the accumulation patterns were the same whether based on specific activity (Fig. 2) or total ^{14}C present in the tissue (Table I).

Accumulation profiles for leaf laminae also showed a distinct age difference. The gradual increase of ^{14}C in mature leaves (LPIs 7–9) during continuous feeding probably reflected xylem movement into the laminae (Fig. 3, A and B). In contrast, ^{14}C rapidly accumulated in young developing leaves. Within 5 min, the concentration of ^{14}C was greater in leaves at LPIs 4 and 5 than in more mature leaves. Within 15 min, the total amount of ^{14}C was greater in LPI 4 (⅓ to ½ expanded) than in LPIs 8 and 12 (fully expanded) (Table I). This accumulation pattern was the same for plants with either 9 or 13 leaves below LPI 0 (Fig. 3, A and B), with method of application (pulse-chase or continuous feeding, natural or artificial xylem sap), with treatment time, and for either specific activity or total ^{14}C present—the patterns of accumulation based on total ^{14}C present in the different-aged leaves simply shifted one or two leaves down the plant compared to those obtained with specific activity (Fig. 3A).

Microautoradiography of Stem and Leaf Vasculature. Microautoradiographs of the older stem internodes (*e.g.* LPI 13) showed heavy accumulation of ^{14}C in rays, metaxylem parenchyma, and outer cells of the pith with lesser amounts in cambium and cortex (Fig. 4A). Little ^{14}C was present in the phloem at this level in the stem.

By internode LPI 8, accumulation in phloem sieve tubes was noticeable (Fig. 4B). Vascular regions of phloem accumulation were associated with young leaf traces that had small amounts of metaxylem. Older traces with extensive metaxylem showed accumulation in the metaxylem parenchyma and rays but little phloem accumulation. Little ^{14}C was retained in the cambium, although it was incorporated into slightly older differentiating cells in both xylem and phloem (Fig. 4, A and B).

By internode LPI 7, most of the [^{14}C]glutamine was found in the phloem (Fig. 5). Note the heavy accumulation of ^{14}C in phloem of traces that lay near the departing central (7C) and left (7L) traces of LPI 7 (Fig. 5, A and B). These traces lead to the young developing leaf at LPI 4. Particularly heavy concentrations of ^{14}C were present in 4C and its bundle-split (4Cs) (18). Little activity was present in either the xylem or phloem of the departing LPI 7 traces, indicating both little xylem movement into and phloem retransport from this mature leaf. Most of the glutamine had been transferred from the LPI 7 traces to traces serving younger leaves in the subtending internode before the traces of LPI 7 had completely diverged from the vascular cylinder. Farther up the stem in the internode (Fig. 4C) and petiole (Fig. 4, D and E) of the developing leaf at LPI 3, almost all of the ^{14}C from glutamine had been transferred to the phloem for translocation to the young developing leaves.

DISCUSSION

Young cottonwood leaves (LPIs 0–5) are rapidly expanding and require large amounts of carbon, nitrogen, and other nutrients. Although developing leaves photosynthetically fix CO_2 (3, 4), most carbon and all of the nitrogen is imported via the xylem and phloem. The objective of this study was to determine the distribution of xylem-borne nutrients within shoots of cottonwood by following the movement of [^{14}C]glutamine in xylem, between xylem and phloem, and to developing leaves and stems.

Organic nitrogen compounds moving in the xylem should initially accumulate in recently matured, fully expanded leaves that are responsible for most transpirational water loss (1, 10). In both lupin (9) and pea (15) fed either [^{14}C]glutamine or [^{14}C]-asparagine via the xylem, much of the ^{14}C initially entered the recently matured leaves. Autoradiography (not shown) and the total accumulation of ^{14}C in mature and developing cottonwood leaves showed that little xylem-fed glutamine moved into the mature leaves (Table I). Rather, it moved directly upward in xylem or from xylem to phloem in the stem and then accumulated in young developing leaves (Fig. 3). This accumulation was closely correlated with the sink strength of the leaf (LPIs 2, 3, and 4 are the most rapidly expanding leaves and are the strongest sinks in the developing leaf zone). The accumulation of ^{14}C in leaves approaching maturity (LPIs 5 and 6) may reflect either a continuing demand for amino acids or an immature xylem to phloem transfer system. Thus, more glutamine would move into the lamina with the transpiration stream. ^{14}C that did accumulate in the mature leaves is probably a measure of the glutamine that bypassed the transfer system in the stem (Table I). We found no evidence that ^{14}C from glutamine was retranslocated from matures leaves (see Fig. 5). Microautoradiography of mature petiole cross-sections indicated no phloem loading and retransport of glutamine as was found with aspartic and glutamic acid (17).

Removing several mature leaves from the lower stem decreased ^{14}C accumulation in xylem and phloem in the stem segments subtending these missing leaves (Fig. 1). When lower leaves were present, ^{14}C accumulated throughout the stem (Fig. 2). These

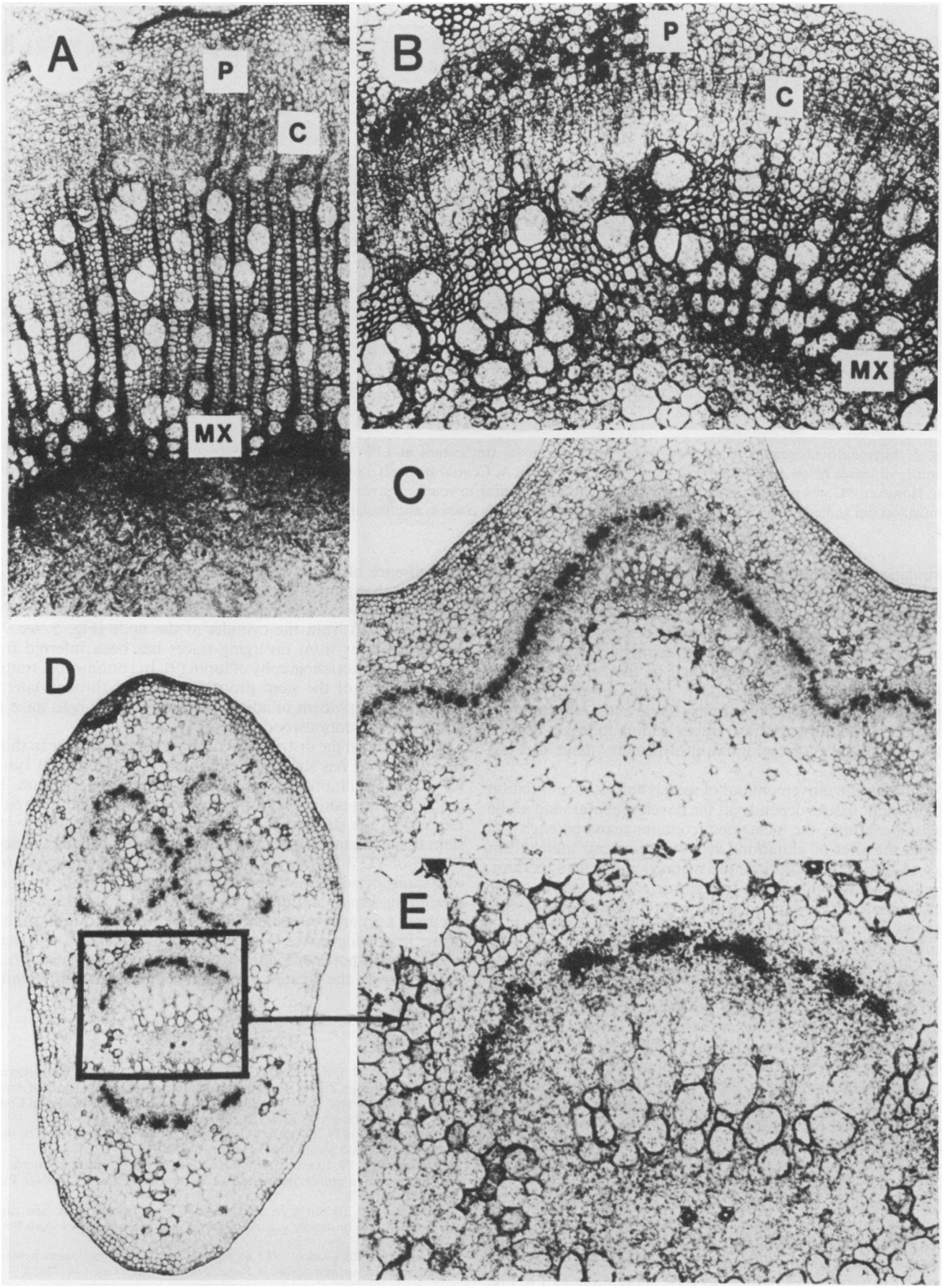

FIG. 4. Microautoradiography of internode and petiole transections after xylem feeding [U-^{14}C]glutamine. Treatment, 1 h (15 min pulse, 45 min chase). Exposure, 8 d for A, 40 d for B to E. A, Internode at LPI 13 showing ^{14}C concentrated in metaxylem parenchyma (MX), rays, and outer pith, but less ^{14}C in either phloem (P) or cambium (C), (× 89). B, Internode at LPI 8 showing absence of phloem accumulation and heavy metaxylem accumulation in the trace serving an older leaf (right) and active phloem accumulation with little metaxylem accumulation in the trace serving a younger leaf (left) (× 142). C, Internode at LPI 3 showing ^{14}C concentrated in the phloem. Phloem labeling was continuous around the entire vascular cylinder (× 71). D, Approximate midpetiole of a leaf at LPI 3 showing ^{14}C concentrated in the phloem of a young importing leaf (× 71). E, Boxed area of D showing heavy label in phloem with some transport of label into surrounding tissue (× 223)

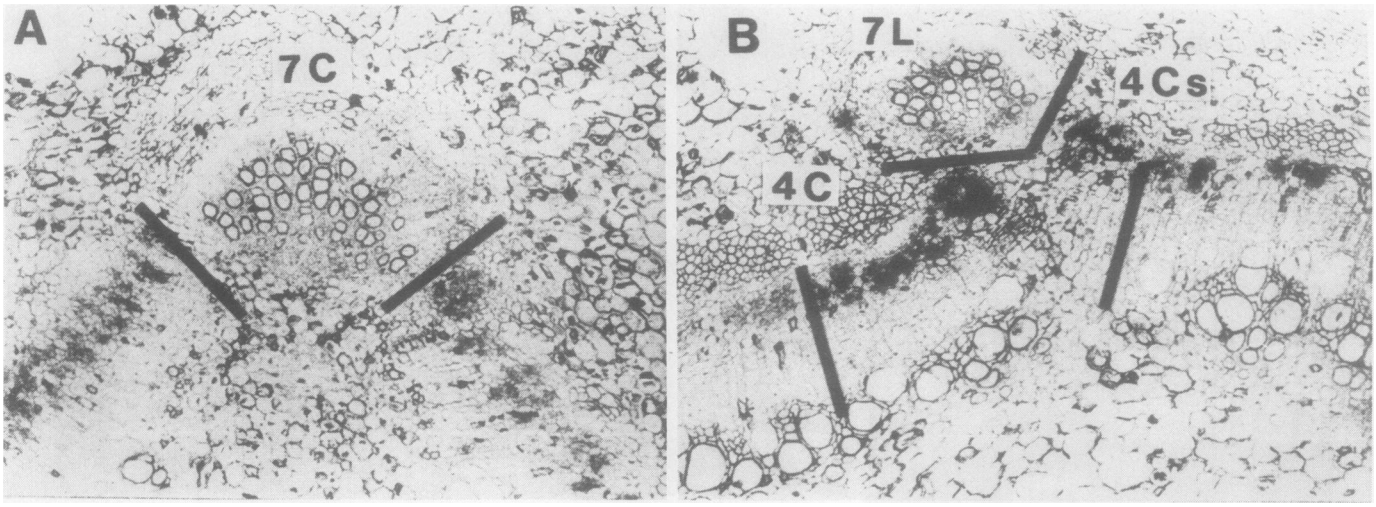

FIG. 5. Microautoradiographs of selected traces from the nodal transection at LPI 7. Treatment, 0.5 h continuous feeding with [U-^{14}C] glutamine, all leaves below LPI 9 removed; exposure, 13 d (× 68). A, Central trace (7C) of a leaf at LPI 7 showing little ^{14}C in either phloem or xylem. However, ^{14}C was present in the phloem of adjacent traces leading to younger leaves. B, Left trace (7L) of a leaf at LPI 7 showing no phloem accumulation but some ^{14}C in the metaxylem parenchyma of 7L. Heavy phloem accumulation is present in 4C and 4Cs, traces leading to a leaf at LPI 4.

accumulation patterns can be explained by considering the amount of primary and secondary xylem tissue associated with the traces leading to mature leaves and how these tissues influence glutamine movement in xylem. When the lower leaves (LPIs 10–13) were removed before treatment, [^{14}C]glutamine moving up the stem was concentrated in the xylem of traces leading to leaves at LPIs 7, 8, and 9. In the lower stem these traces have a small amount of primary tissue and a large amount of secondary xylem. In contrast, traces leading to leaves at LPIs 11, 12, and 13 have a larger amount of primary tissue and less secondary xylem.

[^{14}C]Glutamine movement out of vessels and its accumulation in other xylem tissues depends on the developmental state of the tissue. For example, the metaxylem contains many parenchyma cells that are sites of glutamine accumulation and metabolism (Fig. 4, A and B). In addition, the metaxylem borders the pith, which accumulates glutamine. In contrast, movement out of the vessels of secondary xylem occurred mostly via ray- and vessel-associated parenchyma. The fibers of secondary xylem, which make up most of the tissue, do not accumulate ^{14}C (Fig. 4A). Thus, when glutamine moves in a trace composed largely of secondary xylem, little is accumulated in that tissue. Traces that lead to recently matured leaves at midstem positions, *e.g.* LPI 8, possess mostly primary tissue in their upper levels and mostly scondary tissue in their lower levels (6). Therefore, an accumulation gradient is present that favors both upward movement in xylem and xylem to phloem transfer of glutamine to younger traces leading to developing leaves. In addition, the [^{14}C]glutamine accumulated by metaxylem parenchyma of traces leading to older leaves could be transferred through rays to vessels in the secondary xylem of traces leading to recently matured or developing leaves for movement up the stem.

Microautoradiography shows that the ^{14}C present in phloem of the lower stem was diffused throughout the tissue and not concentrated in sieve tubes (Fig. 4A). Actual loading into sieve tubes first takes place in midstem (*e.g.* see the heavy concentration of ^{14}C in sieve tubes of an internode subtending LPI 8; Fig. 4B). In this internode, ^{14}C in the phloem was concentrated in specific traces leading to young developing leaves. Such traces can be tentatively identified by the small amount of metaxylem present. At the node of LPI 7, the region of the first fully expanded leaf (3), the phloem of LPI 4 traces was heavily labeled. The absence of ^{14}C in LPI 7 traces (Fig. 5, traces 7C, and 7L) indicated that glutamine was transferred from the LPI 7 traces to adjacent traces of young developing leaves before the LPI 7 traces diverged from the cylinder at the node (Fig. 5, see 7L). Similar transfer from diverging traces has been inferred from whole stem autoradiography of lupin (9). In cottonwood, transfer in this region of the stem probably occurred through laterally anastomosing phloem of adjacent traces; transfer cells have not been found in cottonwood nodes (5).

This study on the distribution of xylem-fed glutamine in shoots of cottonwood has shown that the stem has an efficient system for removing glutamine from the transpiration water stream. The amide, with its additional nitrogen complement, was then either retained in the stem where it was stored or metabolized by the stem tissue (metaxylem parenchyma still contained high concentration of ^{14}C 3 h after a 15-min pulse of [^{14}C]glutamine), or it was transported upward in both xylem and phloem to young developing leaves and the shoot apex. Final upward movement occurred primarily in phloem and was a function of sink strength of the importing leaves (Fig. 3; Table I). Glutamine, the major nitrogen transport compound in cottonwood, was thus directed to tissues with the greatest growth-dependent demand for nitrogen.

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