JOURNAL OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE

VOLUME 96 MARCH 1971 NUMBER 2

Translocation of Glutamate-^{14}C and Aspartate-^{14}C by Intact Apple Trees[1]

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Abstract. Glutamate-^{14}C and aspartate-^{14}C were supplied to the roots of 1-year-old MM 106 rooted apple cuttings. Both compounds were readily absorbed and translocated to the aerial parts of the plants. Glutamate, and its metabolic products, tended to accumulate in phloem tissues to approximately the same levels as were detected in the xylem. This was in contrast to aspartate, and its products, which were concentrated in the xylem.

Extensive metabolism occurred during uptake and translocation with detectable level in respiratory CO_2 and in protein. However, emphasis is given to the recovery of labeled amino acids in aerial tissues. This supports previous findings indicating the significance of amino acids as N carriers in the apple.

The amino acids, aspartate and glutamate, and their amides, asparagine and glutamine, contain most of the N of the tracheal sap (xylem) in many species of woody perennial plants (2, 14). Bollard (3) found that 70-90% of the N of apple trachael sap was in these amino acids and amides, with the amides predominant. Under field conditions free nitrate has been detected in very low concentrations only in fine roots and was absent in other tissues. Nitrate has been detected in xylem and phloem in greenhouse studies where nitrate has been applied in nutrient solutions at concentrations above 100 ppm (8). The absence of nitrate in xylem and phloem of the apple has led to the conclusion that nitrate is reduced to ammonia in the roots where the enzyme nitrate reductase has been reported (7). Ammonia is incorporated into amino acids which are then translocated to aerial tissues where they are metabolized to furnish nitrogen to various synthetic pathways. Bollard's results (3), as well as those of Ozerol and Titus (9), indicate that upward translocation of amino acids takes place through the xylem. The evidence is, however, indirect.

Ammonia N is thought to be incorporated into amino acids via transamination of α-keto acids or via the reaction catalyzed by glutamic dehydrogenase (5, 11). Apple and peach roots readily convert organic acids, such as α-ketoglutarate and fumarate, into amino acids, especially glutamic and aspartic (12, 13). It has not been demonstrated that these amino acids, synthesized in the roots, are subsequently translocated to the aerial tissues.

This paper presents the results of experiments using aspartate-^{14}C and glutamate-^{14}C supplied to roots to determine their uptake, gross metabolism, and translocation by 1-year-old intact apple trees.

Materials and Methods

Plant materials. Rooted cuttings of 1-year-old apple trees (*Pyrus malus* L., M. Merton 106) were grown 45 days in nonsterile sand culture. The trees were trained to a single shoot. Eighteen hours before the beginning of an experiment trees were removed from the sand, the roots were rinsed with water, and the trees were placed in an aerated liquid medium, pH 6.0.

Incubation procedure. The roots and basal stem of the pretreated plants were sealed into a widemouthed flask fitted with a slotted rubber stopper sealed with modeling clay. Eight to 10 cm of the stem section below the single leafy shoot protruded above the stopper. The flask contained 250 ml of solution adjusted to pH 6.0 with 50 μc of L-glutamate-^{14}C or L-aspartate-^{14}C, specific activities 14.9 and 8.7 mc/m mole, respectively. Air was pulled through the system by vacuum as described earlier (11) and respired CO_2 was trapped in hyamine hydroxide. Aliquots were removed during the incubation period and counted for ^{14}C in a liquid scintillation spectrometer. Each experiment was run in duplicate.

Analytical methods. At predetermined times a tree was removed from the incubation medium, the roots were rinsed 2 times with deionized water and roots, leaves, bark, and wood were separated and placed in boiling 100% ethanol and boiled for 10 min. The tissues were then homogenized with a Virtis blender and centrifuged at 10,000 x g. The residues were successively extracted 4 times with 80% (v/v) ethanol. The extracts were combined and evaporated to dryness at 35°C in a rotary evaporator.

The soluble extract was then dissolved in water and fractioned sequentially on 1 x 8 cm columns of Dowex 50 (H+) and Dowex 1 (formate) resins (10). The amino acid fraction eluted from the Dowex 50 (H+) column was separated into an acidic amino acid fraction (glutamate and aspartate) and a neutral and basic amino acid fraction by passage over a Dowex 1 (acetate) column. This procedure separates the initial extract into acidic amino acid, other amino acid, organic acid, and sugar fractions. The neutral and basic amino acid fraction was treated with 6N HCl overnight at 100°C to hydrolyze glutamine and asparagine to their respective acids. The hydrolyzates were passed over the Dowex 1 resin to complete the separation. The insoluble residue remaining after centrifugation was also hydrolyzed and then treated in the same manner as the soluble extract. Samples of all the fractions were assayed for radioactivity in the scintillation counter.

The components of the organic acid fraction were separated by paper chromatography in n-butanol:90% formic acid:water v/v/v (1:1:1) aged 24 hr (1). Glutamate and aspartate were

[1]Received for publication August 14, 1970.

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separated by paper chromatography using water-saturated phenol (4). After the chromatograms were dried, the radioactive components were located with a strip scanner.

Results

Both glutamate-^{14}C and aspartate-^{14}C were readily taken up and extensively metabolized by apple roots as evidenced by the quantities of $^{14}CO_2$ given off and the amount of label accumulating in the organic and amino acids (Tables 1 and 2) even in as short a time as 5 min. At the end of 2 hr, one-third of the label initially present in the media as aspartate-^{14}C was recovered as $^{14}CO_2$, whereas approximately the same amount was released from glutamate-^{14}C in 40 min.

Table 1. Appearance of label in CO_2 during incubation of roots of intact apple tree with aspartate-^{14}C and glutamate-^{14}C.

Isotope used	Incubation time in minutes			
	5	30	60	120
			(CPM x 10^{-6})	
Aspartate-^{14}C	0.37	5.63	11.05	30.00
Glutamate-^{14}C	1.40	24.89	38.85	- -

Both aspartate-^{14}C and glutamate-^{14}C were converted into the organic acids of the TCA cycle within 5 min in the roots and the label in the organic acid fraction increased with time. In order of decreasing label, the organic acids labeled were malate>>citrate>α-ketoglutarate≅pyruvate>fumarate>succinate at each time interval, except 60 min when α-ketoglutarate>pyruvate.>citrate>succinate>fumarate. Regardless of the initial source of the isotope, more label was recovered from roots at each time interval in the glutamate plus glutamine fraction than in aspartate plus asparagine fraction (Table 2). The ^{14}C activity of the total amino acid fraction was consistently recovered mainly as glutamate and aspartate and their amides throughout this series of experiments.

The ^{14}C label was rapidly incorporated into the residue fraction of the roots (Table 2). At 5 min the ^{14}C activity of the residue equalled that of the total soluble amino acid fraction. This strong labeling was due to the rapid incorporation of labeled amino acids into proteins which upon acid hydrolysis yielded labeled amino acids.

In the leaves some label was detected within 5 min. However,

Table 2. Distribution of label from aspartate-^{14}C and glutamate-^{14}C in leaves and roots of intact apple trees.

Tissue	Fraction	Incubation time in minutes						
		5		30		60		120
		Asp-^{14}C	Glu-^{14}C	Asp-^{14}C	Glu-^{14}C	Asp-^{14}C	Glu-^{14}C	Asp-^{14}C
					(CPM/g fresh wt x 10^{-3})			
Leaves	All amino acids	0.6	0.2	0.8	0.5	3.6	3.0	12.5
	Aspartate + Asparagine	0.3	0.1	0.5	0.3	0.9	1.5	4.0
	Glutamate + Glutamine	0.2	0.1	0.2	0.1	2.6	1.4	8.4
	Organic acids	0.4	0	0.6	0.4	1.7	1.8	4.9
	Sugars	0.4	0	0.1	0	0.3	0.2	1.0
	Residue	0	0	0	0.1	0.1	0.2	0.7
Roots	All amino acids	75.1	83.9	74.5	167.6	197.0	204.0	127.7
	Aspartate + Asparagine	33.6	10.4	11.4	10.8	65.8	33.7	69.7
	Glutamate + Glutamine	40.7	62.3	54.7	140.6	124.3	152.6	46.4
	Organic acids	28.9	12.8	24.1	29.9	54.4	38.1	63.8
	Sugars	2.8	2.8	7.8	6.9	13.5	11.5	4.1
	Residue	74.0	209.0	97.8	452.0	342.0	886.0	176.0

Table 3. Distribution of label from aspartate-^{14}C and glutamate-^{14}C in bark and wood tissues of intact apple trees.

Tissue	Fraction	Incubation time in minutes						
		5		30		60		120
		Asp-^{14}C	Glu-^{14}C	Asp-^{14}C	Glu-^{14}C	Asp-^{14}C	Glu-^{14}C	Asp-^{14}C
					(CPM/cm x 10^{-3})			
Bark	All amino acids	0.1	0.5	0.2	0.9	0.6	1.3	0.8
	Aspartate + Asparagine	0.1	0.2	0.1	0.3	0.3	0.7	0.4
	Glutamate + Glutamine	0	0.3	0.1	0.5	0.3	0.4	0.4
	Organic acids	0	0	0.1	0.2	0.3	0.5	0.6
	Sugars	0	0	0.1	0	0	0	0
	Residue	0	0	0.2	0.8	0.3	1.2	0.5
Wood	All amino acids	0.1	0.4	0.5	1.2	1.2	1.9	11.1
	Aspartate + Asparagine	0	0.2	0.3	0.6	0.9	0.7	5.6
	Glutamate + Glutamine	0.1	0.2	0.2	0.5	0.3	1.1	3.3
	Organic acids	0	0	0.3	0.3	0.3	1.1	0.3
	Sugars	0	0	0.1	0	0	0	0
	Residue	0	0	1.5	1.0	0.4	1.7	0.3

the amount was low over all time intervals and was concentrated in the amino acid fraction. The total label in the amino acid fraction of the leaves was slightly higher when aspartate-^{14}C was supplied than when ^{14}C was supplied as glutamate. The amino acids aspartate and glutamate and their amides accounted for 36-100% of the total soluble labeled compounds in the leaves over the time periods studied. Some label was detected in sugars, more so when ^{14}C was supplied as aspartate. Very little label was detected in the residue of the leaves after ethanol extraction.

Table 3 gives the amount of label detected in the soluble extracts of wood (xylem) and bark (phloem) tissues of trees supplied with either ^{14}C amino acid. The values are expressed as CPM/linear cm of shoot. Generally, more label was detected in the wood than in the bark. However, even at 5 min, label was detected in both tissues. Unlike the leaves, the residue of both bark and wood did contain label with as much as 30-40% of the total activity detected in that fraction after 30 min. More label in the amino acid fraction was found in the stem tissues when glutamate-^{14}C was the substrate. Wood tissues were labeled to a greater extent than was the bark after 5 min.

Discussion and Conclusions

These experiments indicate that ^{14}C from aspartate and glutamate is readily taken up by the roots of 1-year-old apple trees and is rapidly translocated to the aerial portions of the plant. The amino acid fraction of leaf tissues was labeled within 5 min after ^{14}C-labeled amino acids were supplied to the root systems of experimental plants. Extensive metabolism evidently occurred during the uptake and translocation processes.

In past experiments with α-ketoglutarate and fumarate (12, 13) it has been shown that apple roots have the enzyme systems to convert the carbon skeletons of organic acids to amino acids in less than 3 min. In these experiments with ^{14}C-labeled aspartate and glutamate, the appearance of the label in organic acids within 5 min suggests that these reactions are reversible. In the experiments referred to above, the ^{14}C label from the organic acids accumulated more rapidly in glutamate than in aspartate. This may explain the early labeling of glutamate when ^{14}C was supplied as aspartate (Table 2).

The experiments using aspartate support the view that organic nitrogen is translocated from roots to the leaves through the xylem in that more label was found in the xylem than in the phloem tissues, especially at 30 and 60 min after the beginning of the uptake experiments. It is not clear that the nearly equal labeling of phloem tissues when glutamate-^{14}C was used indicates phloem translocation or whether this reflects accumulation in those tissues through connecting ray cells.

The organic acid fraction of both wood and bark tissues contained some label at 30 and 60 min. At 5 min, label was found only in the amino acid fraction. Either labeled organic acids were being moved up the stem or the amino acids were being metabolized during the translocation process. The latter possibility is more likely considering the time intervals involved and the differences in the ratio of amino acids to organic acids of the roots versus those of the bark or wood. In addition, the presence of labeled amino acids in the insoluble residue of the bark and wood tissue also indicates that metabolism was occurring in the stem section. As early as 5 min after the beginning of treatment, ^{14}C activity in the organic acid fraction of leaves was detected, although label did not accumulate to detectable levels in these compounds extracted from stem tissues. Either their synthesis was negligible in stem tissues, or they moved too rapidly to accumulate.

In the roots, a considerable amount of label was found in the residue which was accounted for by labeled amino acids released from protein after acid hydrolysis. This indicates that the entering labeled glutamate was rapidly metabolized to other amino acids which do not accumulate as such, but instead are rapidly (even at 5 min) used in protein synthesis.

Very little of the label from translocated amino acids was used in protein synthesis in the leaves. Instead, the label accumulated in the sugar fraction. This is in accord with the idea that leaves accumulate C in protein more from CO_2 than from exogenous amino acids (5,6).

In previous papers (12, 13) it was shown that organic acids fed to excised roots are converted rapidly to amino acids. These experiments give evidence that amino acids of the root system are translocated from roots to leaves and suggest that both the xylem and the phloem may be involved, especially in glutamate translocation. Presumably nitrate N is reduced in the roots to furnish ammonia which is combined (by transaminases or glutamic dehydrogenase) to form amino acids.

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