Plant Physiol. (1982) 70, 476–482
0032-0889/82/70/0476/07/$00.50/0

Metabolism and Translocation of Allantoin in Ureide-Producing Grain Legumes[1]

Received for publication February 8, 1982 and in revised form April 21, 1982

CRAIG A. ATKINS, JOHN S. PATE, ANNE RITCHIE, AND MARK B. PEOPLES
Botany Department, University of Western Australia, Nedlands 6009, Western Australia, Australia

ABSTRACT

Transfer of the nitrogen and carbon of allantoin to amino acids and protein of leaflets, stems and petioles, apices, peduncles, pods, and seeds of detached shoots of nodulated cowpea (*Vigna unguiculata* L. Walp. cv. Caloona) plants was demonstrated following supply of [2-^{14}C], [1,3-^{15}N]allantoin in the transpiration stream. Throughout vegetative and reproductive growth all plant organs showed significant ureolytic activity and readily metabolized [2-^{14}C]allantoin to $^{14}CO_2$. A metabolic pathway for ureide nitrogen utilization via allantoic acid, urea, and ammonia was indicated. Levels of ureolytic activity in extracts from leaves and roots of nodulated cowpea were consistently maintained at higher levels than in non-nodulated, NO_3^- grown plants.

[^{14}C]Ureides were recovered in extracts of aphids (*Aphis craccivora* and *Macrosiphum euphorbieae*) feeding at different sites on cowpea plants supplied with [2-^{14}C]allantoin through the transpiration stream or to the upper surface of single leaflets. The data indicated that the ureides were effectively transferred from xylem or leaf mesophyll to phloem, and then translocated in phloem to fruits, apices, and roots.

The ureides, allantoin, and allantoic acid are major nitrogenous products of nitrogen fixation in nodulated cowpea (10) and soybean (14, 18) and apparently provide much of the nitrogen required for protein synthesis and growth during plant development (10, 19). Although extracts of tissues from cowpea and other 'ureide-producing' grain legumes (10, 20) show allantoinase (EC 3.5.2.5) activity, the metabolic pathway utilizing allantoic acid and releasing nitrogen for amino acid synthesis has not been defined. Allantoicase (EC 3.5.3.4) activity was not detectable in extracts of soybean seedlings (9), and even though urease (EC 3.5.1.5) is apparently a constitutive enzyme in many plants, including soybean (see 17), the exact nature of the relationship between ureolytic activity (12) and ureide metabolism has yet to be resolved.

Transport of ureides in xylem from nodules and nodulated root systems (10, 14, 18) has been well documented, but subsequent transfer from the xylem to phloem streams supplying fruits, apices, roots, and developing leaves has not been established. The present investigation uses ^{14}C-, ^{15}N-labeled allantoin to investigate transfer of the nitrogen and carbon of ureides to amino acids and protein, to elucidate sites and metabolic pathways involved in ureide utilization, and, using aphids, to demonstrate involvement of phloem in ureide transport to sites of utilization in cowpea shoots.

[1] Supported by grants from the Australian Research Grants Committee and the United Nations Development Program through a cooperative project with the International Institute of Tropical Agriculture, Ibadan, Nigeria.

MATERIALS AND METHODS

Plant Material. Effectively nodulated legumes were grown in nitrogen-free sand culture (11) in a naturally lighted glasshouse. Cowpea (*Vigna unguiculata* L. Walp. cv. Caloona) and mung bean (*Vigna radiata* L. Wilczek) were inoculated with *Rhizobium* strain CB756, soybean (*Glycine max* L. Merr. cv. Bragg) with a commercial peat *Rhizobium* inoculum (Root Nodule Pty. Ltd., Nitrogerm Group H), and white lupin (*Lupinus albus* L. cv. Ultra) with *Rhizobium* strain WU425 at sowing. Cowpea, soybean, and mung bean were grown with a day/night temperature regimen of 35/25°C and lupin with a regimen of 30/18°C. Non-nodulated cowpeas were grown in the same way as nodulated plants except that seed was not inoculated and the nutrient solution contained 10 mM KNO_3 (2).

Preparation of ^{14}C- and ^{15}N-Labeled Allantoin. Samples of [2-^{14}C]uric acid (1 mM, 50 μCi; Amersham (Australia)) and [1,3-^{15}N]uric acid (3 mM, 96.4 atom % excess ^{15}N; Merck Sharpe and Dohme, Canada) were dissolved in 5 ml of 20 mM Tricine and adjusted to pH 10.0 with NaOH. After adding 0.5 to 1.0 mg of purified urate oxidase (EC 1.7.3.3; Sigma type IV) the mixture was incubated at 30°C for 36 h with constant shaking to ensure aeration. Ion exchange chromatography (10) of aliquots of the incubation mixture showed a high yield of allantoin with usually less than 10% uric acid remaining, and insignificant hydrolysis of allantoin to allantoic acid (Fig. 1). Purity of the double-labeled allantoin fraction (Fig. 1) was further demonstrated by quantitative yield of [^{14}C]allantoic acid following alkaline hydrolysis, and contamination with urea was disproved by absence of $^{14}CO_2$ evolution after treatment with urease. Following incubation, the bulk reaction mixture was passed through a 3 × 0.5 cm Dowex 50, H^+-form column followed by a 3 × 0.5 cm Dowex 1, formate-form column, and the labeled allantoin eluted with water. The treatment removed the Tricine buffer, uricase protein, allantoic acid, uric acid, and an unknown acidic reaction product (Fig. 1). The final allantoin preparations were then neutralized and stored at −20°C.

Metabolism of Xylem-Borne [^{14}C], [^{15}N]Allantoin. Vegetative and reproductive fruiting shoots of nodulated cowpea were cut beneath water and allowed to transpire for 30 min in xylem sap diluted 6-fold with water. The xylem sap had been previously collected as exudate from the root system following decapitation of nodulated cowpea plants identical with those used in the labeling study. Groups of three shoots were transferred to 30 ml of diluted xylem sap containing 2 mM allantoin labeled with 5.4 μCi of ^{14}C and 0.45 mg of ^{15}N. After 24 h shoots were harvested and component organs homogenized in cold 80% (v/v) ethanol. The ethanol extracts were dried, dissolved in water, and washed with petroleum ether to remove pigments. The resulting water extracts were then fractionated into acid and neutral and basic solutes using a Dowex 50 H^+ resin column, and the labeling in allantoin and allantoic acid determined as described previously (1, 10). Basic fractions were separated into component amino acids

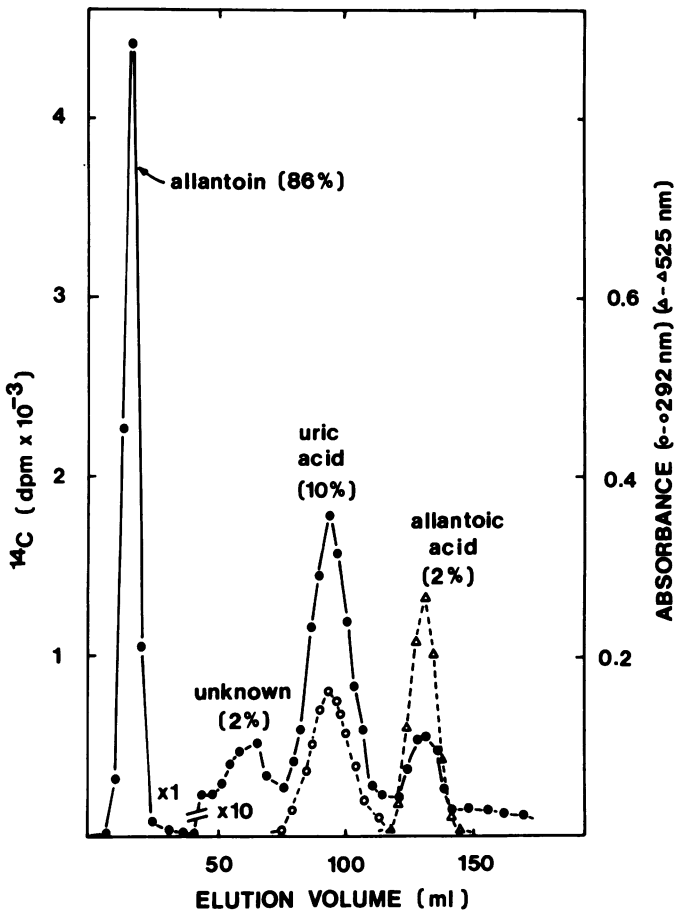

FIG. 1. Ion exchange chromatographic separation of products of [2-^{14}C]uric acid metabolism by urate oxidase. Note that allantoin peak is shown on a scale one-tenth that of following peaks.

using an amino acid analyzer (4). The ethanol insoluble residue was dried and finely milled. ^{14}C in the ethanol soluble fractions, in isolated compounds, or in ethanol insoluble fractions solubilized in hyamine 10 × hydroxide was determined by liquid scintillation, with appropriate corrections for quenching. ^{15}N in extracts, insoluble residues, or isolated compounds was measured by optical emission spectrometry (16) following steam distillation of ammonia from Kjeldahl digests.

Metabolism of [2-^{14}C]Allantoin by Tissue Slices. Freshly harvested, chilled plant material was finely sliced (0.5–1.0 mm strips) and samples (0.1 g fresh weight) added to serum vials containing 2.7 ml of either 50 mM K-phosphate buffer (pH 6.3) or 59 mM Tris-HCl (pH 7.5). The vials were placed under vacuum so that the tissue pieces were visibly 'wetted' and sank to the bottom of the buffer solution. A small tube containing 0.5 ml of 0.1 M NaOH was inserted and after the vials were closed, 0.3 ml of 10 M [2-^{14}C]allantoin (2.2 × 10^4 dpm/μmol) was added through the serum cap. The vials were then incubated in the dark in a shaking bath at 30°C and, after varying periods (30–120 min), the reactions were terminated by adding 0.5 ml of 4 M $HClO_4$. The closed vials were held on ice for a further 2 h to ensure complete absorption of CO_2, opened, and the NaOH removed for ^{14}C measurement by liquid scintillation spectrometry.

Assay of Ureolytic Activity. Extracts of freshly harvested plant tissues were prepared using two volumes of 50 mM Hepes-NaOH (pH 8) containing 5 mM DTT. The homogenate was filtered through 100 μm mesh and the filtrate used as source of enzyme. The reaction mixture comprised 50 mM Hepes-NaOH (pH 8), 1 mM DTT, 5 mM [^{14}C]urea (1.4 × 10^3 dpm/μmol), and 0.5 ml of plant extract in a total volume of 3 ml, contained in a closed, 34-

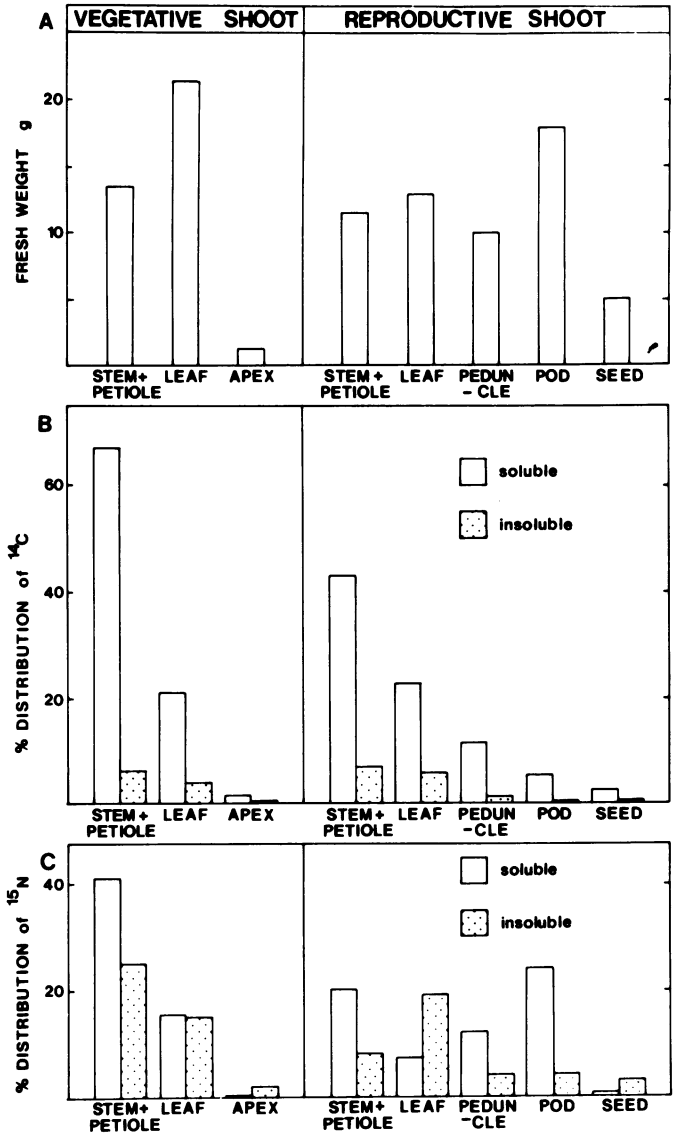

FIG. 2. Fresh weight (A) and distribution of ^{14}C (B) and ^{15}N (C) between organs of cut vegetative or reproductive shoots of nodulated cowpea plants supplied 2 mM [2-^{14}C], [1,3-^{15}N]allantoin (6 × 10^5 dpm of ^{14}C/ml; 48.2 atom % excess ^{15}N) for 24 h in transpiration stream. Soluble and insoluble fractions were separated following extraction in 80% (v/v) ethanol at harvest.

ml serum vial with a small tube containing 0.5 ml of 0.1 M NaOH inserted in the vial to trap released $^{14}CO_2$. After 15 min at 30°C in a shaking bath in darkness, the reactions were terminated and ^{14}C in the trap measured as indicated above.

Labeling Studies using Aphids. Actively growing colonies of the black cowpea aphid (*Aphis craccivora*) and the green potato aphid (*Macrosiphum euphorbiae*) were cultured on cowpea and soybean plants during mid-vegetative and reproductive growth. In a first experiment, aphids were collected from leaflets and fruits 90 or 180 min after placing cut, transpiring reproductive shoots of cowpea in a feeding solution (diluted xylem sap) containing 1 mM [2-^{14}C]allantoin (4 μCi). The labeled solution was completely taken up by the shoots in 20 min and was replaced with diluted, unlabeled xylem sap for the remainder of the feeding period. In a second experiment, the upper surface of a leaflet of the top three trifoliolate leaves of a cowpea plant was wetted with 0.01% (w/v) Triton X-100 followed by 20 μl of 1 mM [2-^{14}C]allantoin (3 μCi, ^{14}C). The plant was held in sunlight and 90 to 110 min after administration of labeled allantoin, groups of feeding aphids (29-

Table I. *Distribution of ^{14}C among Compounds of the Soluble Fraction from Different Organs of Vegetative and Reproductive Shoots of Cowpea*

The organs were supplied 2 mM [2-^{14}C]allantoin (6×10^5 dpm/ml) in the transpiration stream for 24 h.

Plant Part	^{14}C of Soluble Fraction	% ^{14}C of Soluble Fraction as			
		Basic	Acidic and neutral[a]	Allantoin	Allantoic acid
	dpm $\times 10^{-4}$				
Vegetative shoot					
Stem and petioles	296	2.5	55.7	38.1	3.7
Leaflets	53	8.2	75.7	12.3	3.8
Apex	8	4.8	24.5	57.7	12.9
Reproductive shoot					
Stem and petioles	162	2.3	50.9	43.3	3.5
Leaflets	66	4.3	59.6	32.1	3.9
Peduncles	45	2.2	55.3	36.3	6.2
Pods	19	4.2	64.3	29.7	1.8
Seeds	11	2.1	37.5	56.3	4.5

[a] Acidic and neutral compounds other than allantoin and allantoic acid.

Table II. *^{15}N Labeling of the Basic Fraction of the Soluble Extract from Different Organs of Cut Vegetative and Reproductive Shoots of Cowpea*

The organs were supplied ^{15}N as 2 mM [1,3-^{15}N]allantoin (48.2 atom % excess ^{15}N) for 24 h in the transpiration stream.

Plant Part	Total N	^{15}N Excess
	mg	*μg*
Vegetative.shoot		
Stem and petioles	3.57	17.4
Leaflets	3.19	8.4
Apex	1.11	3.2
Reproductive shoot		
Stem and petioles	3.27	2.7
Leaflets	4.84	11.4
Peduncle	1.36	0.7
Fruits	1.93	1.9

93 mg fresh weight) were collected from stems, peduncles, flowers, and fruits. In a third experiment, 4-μCi aliquots of [2-^{14}C]allantoin were applied to the outer 5 mm margin of the upper surface of attached leaflets of flowering plants of cowpea and soybean. After 60 or 90 min, aphids feeding on central parts of the lower surface of the same leaflets were collected, each collected aphid being sited at least 1.5 cm proximal to the outer ring of ^{14}C-labeled leaf tissue.

Structural Study of Aphid Stylets. Feeding aphids were anesthetized by exposure to CO_2 and fixed in 15 mM buffered glutaraldehyde while still attached to the plant material. Examination of the location of stylets in the tissue fragments was made using serial 2-μm sections of resin-embedded tissue stained with toluidine blue (pH 6.5) (see 8).

Analysis of Aphid Extracts. Aphids collected from the ^{14}C-labeled plants were weighed, plunged into ice cold 80% ethanol, and homogenized. The homogenates were then centrifuged and the supernatant evaporated to dryness and redissolved in distilled H_2O. Samples were treated to hydrolyze any allantoin present to urea and glyoxylate (5, 10) and [^{14}C]urea determined by incubating the hydrolysate in a closed serum vial containing 1 ml of 1 M Tris-HCl (pH 8), 1 to 2 units purified jackbean urease (Sigma type VII), and a small vial of 0.1 M NaOH to trap $^{14}CO_2$. After 20 min at 30°C, the reaction was terminated and ^{14}C in the alkali trap measured as above. Any [^{14}C]urea present in the aphid extract was determined in the same way except that the hydrolysis steps were omitted. The [^{14}C]allantoin in aphid extracts was also identified by ion exchange column chromatography (Fig. 1), before and after alkaline hydrolysis of the extract (10).

RESULTS

Utilization of N and C of Xylem-Borne Allantoin. Uptake of [2-^{14}C], [1,3-^{15}N]allantoin by transpiring cut vegetative or reproductive shoots of cowpea resulted in uptake of both labels into soluble and insoluble fractions of all shoot organs (Fig. 2). Extensive metabolism of the allantoin occurred in vegetative shoots. All of the supplied ^{15}N, but only 82% of the ^{14}C, was subsequently recovered in the component organs. In the reproductive shoots, 85% of the ^{15}N and 65% of the ^{14}C were recovered, also indicating significant loss of the carbon of the fed allantoin as CO_2. The distribution of ^{14}C among soluble and insoluble fractions of shoot organs differed markedly from that of ^{15}N, a much greater proportion of the ^{15}N being recovered as insoluble materials, a relatively greater proportion of the ^{14}C remaining in the soluble fractions. The differing fates of the ^{14}C and ^{15}N in the shoot were further demonstrated by comparing the ratios of ^{14}C and ^{15}N recovered in insoluble and soluble fractions of shoot parts with the ratio of the two labels in the allantoin initially supplied in the transpiration stream. The fed allantoin contained 13.2×10^3 dpm/μg of ^{15}N, whereas the equivalent ratios for the soluble fractions ranged from 19.7 to 155.0 (average - 54.9×10^3 dpm of ^{14}C/μg ^{15}N) indicating differential enrichment with the urea carbon of allantoin. Conversely, in the insoluble fractions, $^{14}C/^{15}N$ ratios ranged from 2.1 to 17.7 (average - 5.2×10^3 dpm of ^{14}C/μg ^{15}N)—evidence of differential incorporation of the N rather than the C of allantoin. Enrichment of the insoluble fractions with labeled nitrogen was most marked for actively growing organs (the vegetative apex and developing seeds in Fig. 2), presumably because of high rates of protein synthesis in these parts.

Metabolic Pathway of Allantoin Utilization. Fractionation of the soluble components of extracts from organs of shoots supplied ^{14}C-labeled allantoin in the transpiration stream for 24 h showed that although a significant proportion of the label remained in allantoin, there was extensive transfer to other products (Table I). The labeled allantoic acid found in all tissues could have resulted from allantoin hydrolysis by receptor organs or from hydrolysis in the stem. Similarly, the large proportion of ^{14}C recovered in the acidic and neutral fraction of all organs could have resulted either from metabolism of allantoin *in situ* or from translocation of already formed degradation products from other sites in the shoot. Organs of both shoot types differed markedly in the amount of soluble ^{14}C found in each and in the proportion recovered as allantoin and allantoic acid. The stem and petioles accumulated the greatest amounts of label and showed the highest proportions of ^{14}C as ureides, indicating preferential storage of allantoin. In leaflets, however, especially in those of the younger vegetative

Table III. *Distribution of ^{14}C and ^{15}N among Amino Compounds from the Basic Fraction of the Soluble Extract of the Stem and Petioles and the Leaflets of Cut Vegetative Shoots of Cowpea*

The tissues were supplied 2 mM [2-^{14}C], [1,3-^{15}N]allantoin (6×10^5 dpm/ml, 48.2 atom % excess ^{15}N) for 24 h in the transpiration stream.

Compound	Stem and Petioles			Leaflets		
	Mass	^{14}C content	^{15}N content	Mass	^{14}C content	^{15}N content
	μmol	*dpm × 10^{-3}*	*μg*	*μmol*	*dpm × 10^{-3}*	*μg*
Urea	—	14.4	—[a]	—	2.2	—
Aspartate	6.0	2.2	—	0.5	ND	—
Threonine and serine	12.5	5.3	0.64	12.0	5.5	0.59
Asparagine and glutamate[c]	20.8	15.3	4.82	35.7	8.7	4.40
Glutamine	9.3	5.1	0.92	0.8	1.4	0.01
Proline	13.4	0.3	ND	0.9	1.1	0.02
Glycine	0.8	0.8	—	1.3	0.7	—
Alanine	7.4	4.7	1.80	14.3	1.3	0.51
α-Aminobutyrate	3.6	ND[b]	—	11.5	0.6	—
Arginine	1.6	1.9	—	0.2	ND	—

[a] Not assayed as the small amounts of nitrogen precluded ^{15}N analysis.
[b] Less than 0.1×10^3 dpm or 0.01 μg ^{15}N.
[c] Predominantly asparagine.

Table IV. *Rate of Formation of $^{14}CO_2$ from the Metabolism of [2-^{14}C]Allantoin by Intact Tissue Slices of the Vegetative and Reproductive Parts from Four Legumes*

Species	Young Leaflets	Mature Leaflets	Stems and Petioles	Mature Roots	Root Tips	Stage	Pod	Seed
	nmol/h·g fresh wt							
Vegetative plant parts						Reproductive plant parts		
Vigna unguiculata	93	196	62	33	31	*Vigna unguiculata* Young[b]	116	69
Gycine max	30	187	14	2	9	Maturing[c]	134	36
Vigna radiata	—[a]	238	139	—	—	Mature[d]	—	38
Lupinus albus	—	5	4	—	—	*Glycine max* Young	18	199
						Maturing	61	36
						Mature	—	14
						Vigna radiata Young	10	10
						Maturing	110	18

[a] —, Not assayed.
[b] Early embryo expansion.
[c] Midway through cotyledon filling.
[d] Dry seed at final harvest.

shoot, only a small proportion of incoming allantoin was stored (Table I). Peduncles also apparently functioned in the storage of ureides, whereas in pod tissue there was extensive metabolism to form other solutes. The distinct difference in the proportion of ^{14}C recovered as ureides shown by pods compared with seeds (Table IB) suggests that one function of pods may be to metabolize ureides and transfer the nitrogen to seeds as other compounds.

Labeling of the basic fractions of plant organs with ^{14}C (Table I) indicated that a small amount of the carbon of allantoin had been utilized for amino acid synthesis, and recovery of ^{15}N in these fractions was consistent with the transfer of ureide nitrogen to amino nitrogen (Table II). Organs differed in the amount of ^{15}N found in the basic fraction of each, with the highest values being recorded for petioles of the vegetative shoot and for leaflets of the reproductive shoot (Table II). These basic fractions were further analyzed by separating the component amino acids and measuring both ^{14}C and ^{15}N in the major compounds (Table III).

Most amino acids showed a lower $^{14}C/^{15}N$ ratio (average - 4.8 $\times 10^3$ dpm of $^{14}C/\mu g$ ^{15}N) than the supplied allantoin (13.2) consistent with a more ready incorporation of the ureide N than of the urea C of allantoin in their synthesis. Asparagine was the predominant amino acid of the soluble pools of both organs and in each case was the most heavily labeled, especially with ^{15}N. In general the labeling reflected the abundance of each of the compounds analyzed except for proline, which in stems and petioles occurred at a high level but which contained little ^{14}C or ^{15}N. Small amounts of ^{14}C-labeled urea were recovered from certain tissue extracts (Table III) but the levels of nitrogen in urea were low and precluded assay for ^{15}N.

Application of [2-^{14}C]allantoin to tissue slices of different organs of vegetative and reproductive nodulated plants of cowpea, soybean, and mung bean resulted in release of $^{14}CO_2$ (Table IV). Of the vegetative tissues tested, mature leaflets and young developing fruit tissues were the most active in ureide metabolism. In contrast to the above three ureide-producing species, the leaves and stems of *L. albus*, a species transporting amides but not ureides as nitrogenous solutes of xylem and phloem (4), showed virtually no activity in releasing $^{14}CO_2$ from the labeled allantoin (Table IVA).

Tissue extracts from all organs of nodulated cowpea plants exhibited considerable ureolytic activity during vegetative and early reproductive growth (Fig. 3A). Similar plants cultured on nitrate and not nodulated also showed some ureolytic activity, but enzyme levels were generally lower than in nodulated plants (Fig. 3B). This difference was especially marked for roots and leaves.

Translocation of Allantoin within Shoots. Administration of xylem-borne [2-^{14}C]allantoin as a 20-min pulse in the transpiration

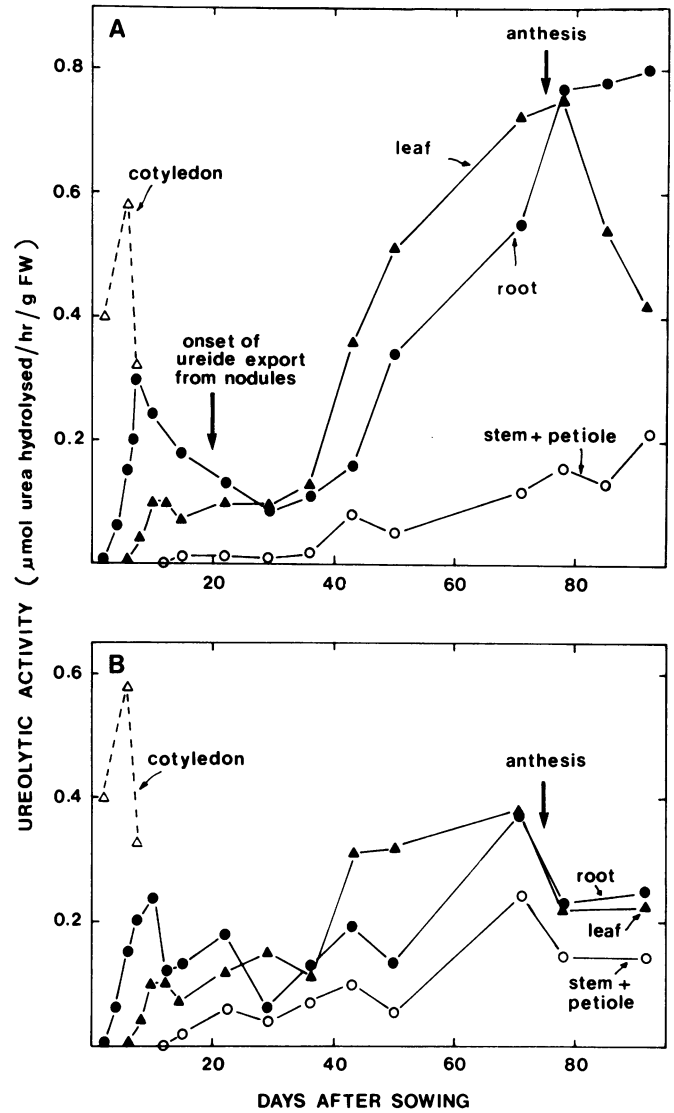

FIG. 3. Distribution of ureolytic activity in extracts of component organs of nodulated (A) or non-nodulated (B) cowpea plants during development. Onset of ureide export from nodules was determined by xylem sap analysis as in a previous study (5). In (A), samples of root tissue extracted for assay excluded nodules.

stream to cut, fruiting cowpea shoots resulted in labeling of the ethanol soluble fraction of aphids feeding on the leaves and subtended fruits (Table V). A significant proportion of the ^{14}C recovered in aphids at both feeding sites at 90 or 180 min was identified as ^{14}C-labeled ureide and at each site the proportion of ^{14}C as ureide increased with time. [^{14}C]urea was not detected or was barely detectable (leaflets 180 min) in the aphids. The identity of labeled solutes other than ureides and urea was not determined.

Labeled allantoin applied to the upper surface of photosynthesizing leaflets of intact plants resulted in transfer of ^{14}C to aphids feeding on the petioles of the fed leaf and on the peduncles, fruits, or flowers subtended by the fed leaves. Labeled carbon was also present in aphids feeding on reproductive organs subtended by a leaf which was not labeled, or on lower parts of the main stem situated some 15 cm below the nearest fed leaflets (Fig. 4). In all cases, extracts of aphids collected 90 min after applying [2-^{14}C]allantoin contained ^{14}C ureide. The total amount of ^{14}C recovered and the proportion of this as ureide (1.5–58.5% of total ^{14}C) varied markedly between sites.

Translocation of allantoin over shorter distances was demonstrated by collecting aphids feeding on veins of the central part of the lower surface of an attached leaf following application of [2-^{14}C]allantoin to the outer margin of the upper surface of the same leaf. In cowpea and soybean leaves after 1 h, ^{14}C ureide constituted 39% to 40% of label recovered from aphids (Table VI). Aphids collected from soybean leaves after a further 30 min contained considerably more ^{14}C, but the proportion as ureide was less, presumably due to more extensive metabolism in the plant or the aphids.

Aphids used in these experiments secreted a copious, sugar-rich, honeydew consistent with their feeding from phloem. Serial sections cut of fixed material embedded in glycol methacrylate indicated that the stylets of the feeding aphids terminated in the phloem of the vascular bundles of fruit or leaf.

DISCUSSION

Based on studies of ureide flow in xylem and ureide and total N accumulation in plant tissues, Herridge *et al.* (10) concluded that allantoin and allantoic acid provide the bulk of the nitrogen required for protein synthesis throughout shoot development of nodulated cowpea. The present study provides direct evidence for the utilization of ureide nitrogen in the synthesis of soluble amino acids and insoluble nitrogen-containing compounds. The demonstration of differential enrichment of amino acid and insoluble fractions of plant organs with the ^{15}N as opposed to the ^{14}C of fed allantoin, indicates a preferential utilization of ureide nitrogen in pathways of nitrogen assimilation in the shoot.

Previous studies found that allantoinase activity is widely distributed in vegetative and reproductive tissues of cowpea (10) and soybean (20). Demonstration in this study of labeled allantoic acid in all organs of cowpea shoots following xylem uptake of [2-^{14}C]allantoin is consistent with such activity. Similarly, detection of [^{14}C]urea in tissue extracts of the same fed shoots suggests an involvement of allantoicase (EC 3.5.3.4) in the cleavage of [^{14}C] allantoic acid. However, urea would also be formed were allantoic acid to be degraded by a mechanism involving allantoic acid amidohydrolase (EC 3.5.3.9) and ureidoglycolase (EC 4.3.2.3), with attendant formation of ammonia, ureidoglycine, and ureidoglycolate (19, 21). Intact tissues of cowpea, especially mature leaflets and stems, were shown here to readily metabolize [2-^{14}C] allantoin to form $^{14}CO_2$, and this, coupled with the capacity of extracts from all tissues of cowpea to hydrolyze urea, indicates that allantoin is metabolized via allantoic acid and urea to ammonia and CO_2 (see also 10, 19, 20). Although the ^{15}N labeling of component amino acids of the soluble pool of both stem and petioles and leaflets (Table III) does not indicate the major route for ammonia reassimilation, the wide range of compounds labeled by [^{15}N]allantoin indicates that ureide nitrogen is readily utilized.

As with seeds of many other legumes (6), the cotyledons of cowpea contain considerable ureolytic activity (Fig. 3). Although ureides constitute a small proportion of total seed nitrogen of cowpea (*e.g.* 4% [10]), the intense hydrolysis of nucleic acids known to occur following the onset of germination of legume seeds (15) might well be a source of purines and ureides (7), whereas the transient increases in ureolytic activity shown here for seedling root and shoot are consistent with these organs engaging in the metabolism of ureides and/or urea translocated from the cotyledons. Thereafter, as the cotyledonary source of nitrogen is progressively utilized, the specific activity of the seedling tissues for urea hydrolysis declines. Although all tissues of cowpea show ureolytic capacity throughout development, activities are particularly high in the root and leaf tissues of nodulated plants following the onset of ureide export to the shoot (see also 5). The high rates of activity in these organs presumably reflect the high requirement for urea metabolism in nodulated plants due to the predominance of ureides as a nitrogen source from the root. In non-nodulated plants grown with nitrate, where ureides have been shown to be present at much lower levels than in effectively

Table V. *Recovery of ^{14}C in Ethanol Extracts from Aphids Feeding on the Leaflets and Fruits of Cut Shoots of Cowpea Supplied 4 μCi (2-^{14}C)Allantoin in the Transpiration Stream in the Light*

The labeled solution was taken up by the shoots within 20 min and was replaced by distilled H_2O for a further 70 or 160 min before the aphids were harvested. Shoots had two mature leaves each subtending two well developed fruits.

Feeding Time	Site of Aphid Collection	Fraction of Aphid Extract	^{14}C Content	
min			*dpm × 10^{-3}/100 mg aphid*	
90	Fruit	Total	2.79	
		Urea	ND[a]	
		Ureide[b]	0.38	(13.7)[c]
90	Leaflets	Total	3.53	
		Urea	ND	
		Ureide	1.40	(39.7)
180	Fruit	Total	9.03	
		Urea	ND	
		Ureide	2.47	(27.4)
180	Leaflets	Total	12.36	
		Urea	0.04	(0.3)
		Ureide	5.88	(47.8)

[a] ND, not detectable.
[b] Allantoin plus allantoic acid.
[c] Values in parentheses are percentage of total ^{14}C as allantoin or urea.

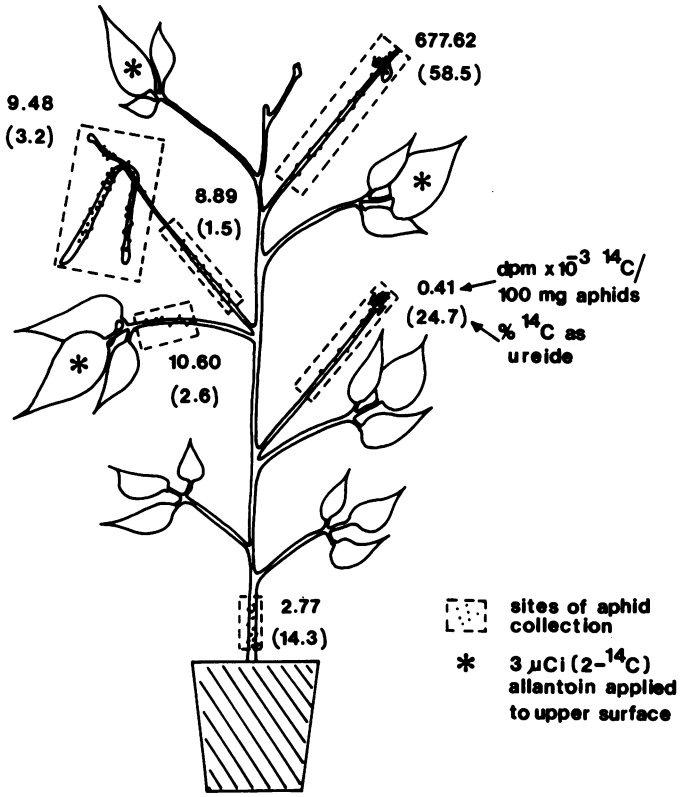

FIG. 4. Recovery of ^{14}C and proportion as ^{14}C ureide (allantoin and allantoic acid) in extracts of aphids collected from feeding sites on an intact, nodulated cowpea plant supplied 3 μCi [2-^{14}C]allantoin to each three leaflets of uppermost trifoliolates. Aphids were collected 90 to 110 min after application of labeled substrate to upper leaflet surfaces.

nodulated plants (2), ureolytic activity proved to be lower in root and leaves. Similar variations in urease activity in response to the presence or absence of added urea have been described for a number of plants (see 17).

This study demonstrates the recovery of ^{14}C ureide as a significant proportion of the total ^{14}C in ethanolic extracts of aphids feeding on fruits, flowers, leaflets, peduncles, and stems, following application of [2-^{14}C]allantoin to the upper surface of leaflets (Table VI, Fig. 4). It is concluded that ureide, as allantoin or allantoic acid, is effectively loaded onto phloem streams moving upward to fruits or downward to the root system. The parallel observations on the fate of transpirationally fed allantoin indicate that ureides are also exchanged freely from xylem to phloem probably using similar mechanisms and routings to those suggested for xylem-to-phloem transfer of amides in *L. albus* (3, 4, 13).

Due to metabolism and utilization of ureides within the aphid, the proportion of ^{14}C recovered as allantoin and allantoic acid at any site is likely to be a minimum value and is likely to underes-

Table VI. *Recovery of ^{14}C in Ethanol Extracts from Aphids Feeding on the Lower Epidermis of Attached Cowpea and Soybean Leaflets on Which 4 μCi (2-^{14}C)Allantoin Was Applied to the Periphery of the Upper Epidermis*

Plants were kept in the light and aphids collected 60 or 90 min after applying the labeled solution. All collected aphids were feeding from the midregion of the leaflet at least 1.5 cm proximal to the upper labeled leaf margin.

Species	Feeding Time	Fraction of Aphid Extract	^{14}C Content	
	min		*dpm × 10^{-3}/100 mg aphid*	
Cowpea	60	Total	13.17	
		Urea	ND[a]	
		Ureide[b]	5.08	(39.0)[c]
Soybean	60	Total	6.29	
		Urea	ND	
		Ureide	2.57	(40.4)
	90	Total	89.39	
		Urea	0.15	(0.2)
		Ureide	4.58	(5.1)

[a] ND, not detectable.
[b] Allantoin plus allantoic acid.
[c] Values in parentheses are the percentage of total ^{14}C as allantoin or urea.

timate seriously the ureide content of the translocation stream. Clearly, too, ureides are readily metabolized by stems and petioles and leaflets. Thus, depending on the relationship between the source organ and the collection site and on the residence time of the labeled phloem sap within the aphids prior to collection, the wide variation in the ^{14}C ureide content of aphids found in the feeding experiments could be expected. The high values (58.5%, Fig. 4) indicate, however, that not only are ureides mobile in phloem, but that they may constitute a significant source of translocated nitrogen for protein synthesis in phloem-fed organs.

Acknowledgments—Skilled technical assistance of E. Raisins, M. Sartori, D. Waldie, and L. Owen is gratefully acknowledged.

LITERATURE CITED

1. ATKINS CA, DT CANVIN 1971 Analysis of ^{14}C-labeled acidic photosynthetic products by ion-exchange chromatography. Photosynthetica 5: 341–351
2. ATKINS CA, JS PATE, GJ GRIFFITHS, ST WHITE 1980 Economy of carbon and nitrogen in nodulated and non-nodulated (NO_3-grown) cowpea (*Vigna unguiculata* L. Walp.). Plant Physiol 66: 978–983
3. ATKINS CA, JS PATE, DL MCNEIL 1980 Phloem loading and metabolism of xylem-borne amino compounds in fruiting shoots of a legume. J Exp Bot 31: 1509–1520
4. ATKINS CA, JS PATE, PJ SHARKEY 1975 Asparagine metabolism—key to the nitrogen nutrition of developing legume seeds. Plant Physiol 56: 807–812
5. ATKINS CA, R RAINBIRD, JS PATE 1980 Evidence for a purine pathway of ureide synthesis in N_2-fixing nodules of cowpea (*Vigna unguiculata* L. Walp.). Z Pflanzenphysiol 97: 249–260
6. BOLLARD EG 1959 Urease, urea and ureides in plants. *In* Utilisation of Nitrogen and its Compounds by Plants. Symp Soc Exp Biol, Vol 13. Cambridge University Press, Cambridge, pp 304–328
7. FUJIHARA S, M YAMAGUCHI 1978 Effects of allopurinol (4-hydroxypyrazolo (3,4-d) pyrimidine) on the metabolism of allantoin in soybean plants. Plant Physiol 62: 134–138
8. GUNNING BES, JS PATE, LW GREEN 1970 Transfer cells in the vascular system of stems: taxonomy, association with nodes, and structure. Protoplasma 71: 141–147
9. HANKS JF, C CHIA, NE TOLBERT, KR SCHUBERT 1981 Metabolism of ureides in soybean seedlings. Plant Physiol 57: S-79
10. HERRIDGE DF, CA ATKINS, JS PATE, RM RAINBIRD 1978 Allantoin and allantoic acid in the nitrogen economy of the cowpea (*Vigna unguiculata* L. Walp.). Plant Physiol 62: 495–498
11. HOCKING PJ, JS PATE 1978 Accumulation and distribution of mineral elements in the annual lupins, *Lupinus albus* L. and *Lupinus angustifolius* L. Aust J Agric Res 29: 267–280
12. KERR PS, BJ RAPP, DG BLEVINS, DD RANDALL 1981 *In vivo* ureolytic activity in soybean leaves. Plant Physiol 67: S-78
13. LAYZELL DB, JS PATE, CA ATKINS, DT CANVIN 1981 Partitioning of carbon and nitrogen in the nutrition of root and shoot apex in a nodulated legume. Plant Physiol 67: 30–36
14. MCCLURE PR, DW ISRAEL 1979 Transport of nitrogen in the xylem of soybean plants. Plant Physiol 64: 411–416
15. OOTA Y, R FUJII, S OSAWA 1953 Changes in chemical constituents during the germination stage of a bean, *Vigna sesquipedalis*. J Biol Chem 40: 649–661
16. PERSCHKE H, EA KEROE, C PROKSCH, A MUEHL 1971 Improvements in the determination of nitrogen-15 in the low concentration range by emission spectroscopy. Anal Chim Acta 53: 459–463
17. SKOKUT TA, P FILNER 1980 Slow adaptive changes in urease levels of tobacco cells cultured on urea and other nitrogen sources. Plant Physiol 65: 995–1003
18. STREETER JG 1979 Allantoin and allantoic acid in tissues and stem exudate from field-grown soybean plants. Plant Physiol 63: 478–480
19. THOMAS RJ, LE SCHRADER 1981 Ureide metabolism in higher plants. Phytochemistry 29: 361–371
20. THOMAS RJ, LE SCHRADER 1981 The assimilation of ureides in shoot tissues of soybeans. I. Changes in allantoinase activity and ureide contents of leaves and fruits. Plant Physiol 67: 973–976
21. VOGELS GD, C VAN DER DRIFT 1976 Degradation of purines and pyrimidines by microorganisms. Bact Rev 40: 403–468