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Xanthine Degradation and Related Enzyme Activities in Leaves and Fruits of Two *Coffea* Species Differing in Caffeine Catabolism

Angela Pierre Vitória and Paulo Mazzafera*

Departamento de Fisiologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, CP6109, 13083-970 Campinas, SP, Brazil

The degradation of xanthine was studied in young and aged leaves and in immature and mature fruits of *Coffea arabica* and *Coffea dewevrei*, which differ with respect to caffeine catabolism. Radioisotope feeding experiments showed that leaves degraded xanthine more readily than fruits but that mature fruits and aged leaves were less efficient than younger tissues. In all cases, a significant part of the recovered radioactivity was in the ureides. Xanthine dehydrogenase was characterized as the enzyme responsible for xanthine degradation, and its activity and that of uricase were consistent with the results obtained in the radioisotope feeding experiments. Activities of allantoinase and allantoate amidohydrolase could not be detected. Considerable levels of endogenous allantoin and allantoic acid were found in fruits and leaves. Therefore, ureide accumulation might be a consequence of low enzyme activity. There was no positive correlation between urease activity and the data from the radioisotope feeding experiments.

Keywords: *Allantoic acid; allantoin; Coffea arabica; Coffea dewevrei; uric acid; xanthine.*

INTRODUCTION

Kalberer (1965) was the first to show caffeine (1,3,7-trimethylxanthine) catabolism in coffee (*Coffea arabica*), using ring- and methyl-labeled caffeine to feed leaves. Although degradation was low, he was able to show accumulation of radioactivity in the ureides allantoin and allantoic acid and in three other unknown compounds. Because of the low degradation rate of this species, further feeding experiments were carried out with labeled caffeine and other methylxanthines (theophylline and theobromine) and xanthine to investigate the caffeine catabolic route in fruits and leaves of coffee (*C. arabica*) and leaves of tea (*Camellia sinensis*) (Suzuki and Waller, 1984a,b; Ashihara et al., 1996). These studies indicated that the main alkaloid degradation pathway might follow the sequence caffeine → theophylline (1,3-dimethylxanthine) → 3-methylxanthine → xanthine → uric acid → allantoin → allantoic acid → urea + glyoxylic acid → NH_4^+ + CO_2. Because of a substantial caffeine degradadation rate, most of these compounds (caffeine to xanthine) could be detected in feeding experiments with fruits and leaves of *Coffea dewevrei* using only tritiated caffeine (Mazzafera et al., 1991; Mazzafera, 1993). In addition, it was observed that an alternative route for caffeine demethylation via theobromine (3,7-dimethylxanthine) → 3-methylxanthine → xanthine was more effective in this coffee species (Mazzafera et al., 1991, 1994a).

From radioisotope feeding experiments, Suzuki and Waller (1984a,b) concluded that the biodegradation of caffeine to dimethylxanthines (theophylline and theobromine) was slow and might be the rate-limiting step in fruits of *C. arabica*. On the other hand, xanthine degradation was observed to occur very rapidly in coffee fruits (Suzuki and Waller, 1984a,b) and tea leaves (Suzuki and Takahashi, 1975).

In fruits (Suzuki and Waller, 1984b) and leaves (Ashihara et al., 1996) of *C. arabica* fed with [2-^{14}C]-xanthine, although a considerable amount of radioactivity was detected in CO_2, there was a significant accumulation of the recovered radioactivity in allantoin and allantoic acid, compared to that observed for urea. In any case, radioactivity was not found in uric acid, indicating a very high turnover. Uric acid was also not detected in tea shoot tips fed with labeled xanthine (Suzuki and Takahashi, 1975).

This paper reports the degradation of [2-^{14}C]xanthine in fruits and leaves of two coffee species differing in their ability to catabolize caffeine. One of them, *C. dewevrei*, converts caffeine more readily to other methylxanthines than the other, *C. arabica* (Mazzafera et al., 1991). The activities of the enzymes operating in this pathway were also investigated for the first time.

MATERIALS AND METHODS

Plant Material. Leaves of the first and fifth pair as well as mature and immature fruits were collected from plants of *C. dewevrei* and *C. arabica* var. Mundo Novo growing in a living collection at the Agronomic Institute, Campinas, Brazil. The *C. dewevrei* plant was the same used by Mazzafera and co-workers in their studies (Mazzafera et al., 1991, 1994a). Immature fruits had soft ivory and opaque endosperm, whereas in mature fruits the endosperm was becoming harder. Endosperms accounted for 36.7 and 47.3% of total fresh mass of the immature and mature fruits of *C. arabica* and for 23.7 and 36.6% for *C. dewevrei*, respectively.

Production of ^{14}C-Labeled Allantoin, Allantoic Acid, and Uric Acid. [2-^{14}C]Allantoin, [2-^{14}C]allantoic acid, and [2-^{14}C]uric acid acid were produced as byproducts of the incubation of [2-^{14}C]xanthine (specific activity = 52.4 mCi/mmol; Sigma, St. Louis, MO) with desalted protein extracts from nodules obtained from soybean plants inoculated with *Bradyrhizobium japonicum* (Woo et al., 1980). Unlabeled

* Author to whom correspondence should be addressed (fax +55 19 289 3124; e-mail pmazza@obelix.unicamp.br).

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allantoin and uric acid were included (0.5 mM) in the reaction mixture. After incubation, the reaction mixture was centrifuged and the supernatant was passed through a Dowex 50-X8-H^+ column (1 mL bed volume), and allantoic acid and uric acid were eluted with distilled water. Xanthine and allantoin were eluted with 4 M NH_4OH. The eluates were dried under reduced pressure, and the compounds in both fractions were further purified by RP-HPLC using 64 mM $(NH_4)_2SO_4$ as isocratic solvent, with a flow rate of 1 mL/min. The peaks identified with a UV monitor (210 nm for allantoin and allantoic acid and 270 nm for xanthine and uric acid) were collected, lyophilized, dissolved in a small volume of water, and chromatographed again, now using water as solvent. From four incubations the yields were 70, 10, and 15% for allantoin, allantoic acid, and uric acid, respectively. Several batches were prepared, and in general the specific activities were low, ranging from 0.3 to 0.7 μCi/mmol.

Radioisotope Feeding Experiments. Immature and mature fruits of both species were fed with [2-^{14}C]xanthine as described by Mazzafera et al. (1991). Ten fruits received the labeled xanthine diluted 2-fold in 50 mM sodium phosphate buffer, pH 6.5. After 48 h of incubation under continuous irradiation (200 μmol m^{-2} s^{-1}), they were frozen in liquid nitrogen, reduced to a powder with a mortar and pestle, transferred to sealed cap tubes, and extracted with 20 mL of 25 mM H_2SO_4 in a boiling water bath for 30 min, with the addition of 500 mg of MgO (Suzuki and Waller, 1984a). The extracts were centrifuged and the supernatants partitioned with chloroform (3 × 20 mL). The aqueous phase was dried under reduced pressure, solubilized in 3 mL of distilled water, and passed through C_{18} Sep-Pak cartridges (Millipore−Waters Associates) that had been previously equilibrated with methanol and water. The first eluting volume and a further washing with 2 mL of 50% methanol (v/v) were combined and dried under reduced pressure. The extracts were solubilized in 2 mL of distilled water, filtered in 0.22 μm membranes, and kept at −20 °C until analysis.

Leaves from the first and fifth expanded leaf pairs were rinsed in tap and distilled water and blotted dry, and disks (2 cm in diameter) were obtained with a cork-borer. Ten disks were immediately weighed and transferred to 25 mL Erlenmeyer flasks containing [2-^{14}C]xanthine diluted in 5 mL of 25 mM sodium phosphate buffer, pH 7.5, and infiltrated for 5 min under vacuum. The disks were then quickly washed in distilled water, blotted dry, weighed, and transferred to a plastic box containing a sheet of foam wet with water at the bottom. The box was covered with glass and left for 48 h under continuous irradiation (200 μmol m^{-2} s^{-1}), and then the disks were frozen in liquid nitrogen and extracted as indicated for fruits. The difference in weight before and after infiltration allowed a rough estimation of the amount of [2-^{14}C]xanthine infiltrated in each disk.

[2-^{14}C]Xanthine Metabolites Analysis. [2-^{14}C]Xanthine metabolites were separated by HPLC using a reversed-phase column (Bio-Sil C18 HL 90-5 S, 250 × 4.6 mm, Bio-Rad). Isocratic chromatography was carried out with 0.5% aqueous acetic acid (v/v), delivered by a Shimadzu pump (model LC 10AS) at a flow rate of 0.5 mL/min. Prior to the analysis, 10 μg of xanthine, 10 μg of allantoin, 10 μg of allantoic acid, and 10 μg of uric acid were added to the samples from the radioisotope feeding experiments. The compounds eluting from the column were monitored with a Shimadzu UV detector (SPD-10A) using 270 nm for detection of xanthine and uric acid and 210 nm for allantoin and allantoic acid. Under these chromatographic conditions the retention times for these compounds were 23.2, 14.7, 5.1, and 5.5 min, respectively. The peaks were collected in scintillation vials, dried at 60 °C, and the radioactivity was estimated in a scintillation counter after addition of scintillation fluid. In view of the very close retention times of allantoin and allantoic acid, their peaks were collected together.

Determination of Endogenous Allantoin and Allantoic acid. The ureides allantoin and allantoic acid were determined in the coffee tissues by measuring glyoxylic acid after acid and basic hydrolysis (Vogels and van der Drift, 1970). Due to interference of phenolic acids we tested different extracting solutions, the most suitable being a cold solution (4 °C) of 200 mM sodium phosphate buffer, pH 7.0, containing 4% PVP 360000 (w/v), at the proportion of 200 mg/5 mL. The plant materials were frozen with liquid nitrogen and ground in a mortar with this solution, and after 30 min at 4 °C, 1.25 mL of aqueous saturated $(NH_4)_2SO_4$ was added. The gum formed was eliminated by centrifugation (27000*g*, 30 min at 4 °C) and the supernatant used for ureide determination. To eliminate the possibility of phenol interference during the color development in the colorimetric assay of glyoxylate, three known amounts of allantoin and allantoic acid were added to the extracts before the hydrolyses were carried out. These results were compared with those obtained with the extracts and pure allantoin and allantoic acid, to check the linearity of the measurements made with extracts supplemented with ureides. This proved that our data were reliable. However, we have also tried to measure ureides using RP-HPLC, but, because several compounds in the extracts absorb in the UV wavelength used for detection (210 nm), strong interferences were observed and the assay had to abandoned.

Protein Extraction and Enzyme Assays. Xanthine oxidase/xanthine dehydrogenase (EC 1.1.3.22, EC 1.1.1.204), uricase (EC 1.7.3.3), allantoinase (EC 3.5.2.5), allantoate amidohydrolase [allantoate amidinohydrolase (decarboxylating)] (EC 3.5.3.9)] and urease (EC 3.5.1.5) activities were investigated in fruits and leaves at the same developmental stages used in the radioisotope feeding experiments. Proteins were extracted from leaves and fruits essentially as described by Mazzafera et al. (1994b). The protein concentrations in the extracts were determined with a ready-to-use reagent from Bio-Rad (Bradford, 1976).

Initial trials with aerated buffer showed that for both coffee species, NAD^+ was essential for activity of the enzyme related with xanthine degradation. This was confirmed by activity staining of protein extracts subjected to native polyacrylamide gel electrophoresis. Therefore, from now on this enzyme will be referred to as xanthine dehydrogenase. Protein extracts were added to the reaction buffer of 50 mM sodium phosphate, pH 7.5, containing 0.2 mM NAD^+, 2 mM Na_2MoO_4, 13.8 mM xanthine, and 0.2 μCi of [2-^{14}C]xanthine (final concentrations). Molybdenum was essential for activity, and preliminary tests showed that unlabeled xanthine increased the enzyme activity. The final reaction volume was 1 mL. The reaction mixtures were incubated at 30 °C for 30 min and then were immediately frozen in liquid nitrogen for further analysis by HPLC of the labeled uric acid formed. Uric acid (5 μg) was added to the reactions, and the radioactivity in the corresponding peak after separation by RP-HPLC was determined as described above. The formation of allantoin in the incubation mixtures was not detected.

For uricase, protein extracts were applied to PD-10 columns (Pharmacia) and eluted with 1 M 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) buffer, pH 10 (Hanks et al., 1981). The reaction mixture contained 0.1 mM uric acid and 0.2 μCi of [2-^{14}C]uric acid, and the final volume was adjusted to 500 μL with 1 M CHAPS buffer, pH 10. Preliminary tests showed that inclusion of unlabeled uric acid increased the activity. The mixture was incubated at 30 °C for 30 min and the reaction stopped by the addition of 50 μL of 0.6 M trichloroacetic acid. Appearance of labeled allantoic acid was determined by collecting the peak separated by HPLC and further radioactivity counting.

Because we did not detect allantoinase and allantoate amidohydrolase activities, the enzyme assays will not be described here. However, basically the methods used by Lukaszewski et al. (1992) were followed.

An in vivo assay was used to determine urease in coffee leaves (Benchemsi-Bekkari and Pizelle, 1992). Ten disks (1 cm in diameter) were obtained with a cork-borer and immediately immersed in cold (4 °C) 100 mM potassium phosphate buffer, pH 7.5, containing 1% ascorbic acid. Fifteen minutes later the disks were quickly rinsed with cold (4 °C) distilled water and transferred to small flasks with 1.5 mL of the same buffer supplemented with 0.1% Triton X-100, 250

Table 1. Percent Distribution of the Recovered Radioactivity from Fruits and Leaf Disks of *C. arabica* and *C. dewevrei* Fed with [2-^{14}C]Xanthine and Incubated for 48 h[a]

infiltrated tissue	applied radioactivity per disk or fruit (μCi)	recovered radioactivity (%)	distribution of recovered radioactivity (%)		
			xanthine	uric acid	ureides
leaf pair					
C. arabica					
1st	0.024	2.2	ND[b]	ND	27.7
5th	0.031	14.2	ND	ND	9.5
C. dewevrei					
1st	0.023	8.1	ND	ND	38.39
5th	0.054	34.6	ND	ND	13.3
fruits					
C. arabica					
immature	0.57	72.8	0.38	0.24	14.94
mature	0.57	76.3	0.72	0.97	15.59
C. dewevrei					
immature	0.55	17.5	11.0	3.05	74.37
mature	0.55	26.9	11.9	13.4	42.99

[a] All data are means of two replicates. [b] ND, radioactivity not detectable.

mM urea, and 3.2 μCi of ^{14}C-urea (specific activity = 58.9 mCi/mmol, Amersham). The disks were infiltrated with this solution under vacuum for 2 min, and then the flasks were sealed with rubber stoppers, with a strip of filter paper wet with hyamine suspended inside the flask. The flasks were incubated at 30 °C for 1 h and then were immersed in liquid nitrogen for disruption of the tissues; the flasks were thawed at room temperature, and 100 μL of 5 M H_2SO_4 was injected with a syringe to release the $^{14}CO_2$ generated by urea degradation. The radioactivity of the $^{14}CO_2$ captured by the filter paper strip was determined in a scintillation counter after addition of scintillation fluid.

Urease activity of fruits was assayed in the protein extracts after the buffer had been changed to 100 mM Tris-HCl, pH 8.0, by filtration through PD-10 columns (Kerr et al., 1983). The assay was carried out in sealed test tubes, and the reaction mixture contained 100 mM Tris-HCl, pH 8.0, 30 mM EDTA, 100 mM urea, and 0.2 μCi of ^{14}C-urea. Preliminary tests showed that the inclusion of unlabeled urea increased the activity. After 1 h at 30 °C, 100 μL of 5 M H_2SO_4 was added and the released $^{14}CO_2$ captured by hyamine by a filter paper strip suspended inside the tube. Radioactivity was determined in the filter paper after addition of scintillation fluid.

RESULTS

The results of the radioisotopic feeding experiments with [2-^{14}C]xanthine, presented in Table 1, show that labeled xanthine was readily degraded in coffee tissues, leaves being more effective than fruits. Although we did not estimate radioactivity in CO_2, the low xanthine recoveries are in agreement with previous studies with coffee leaves and fruits (Suzuki and Waller, 1984a,b; Ashihara et al., 1996), which unequivocally showed that most of the radioactivity of the labeled xanthine was recovered in CO_2.

In addition to the lower xanthine recoveries, the higher degradation rate of leaves of both species compared to fruits was demonstrated by the detection of radioactivity being restricted to the ureides allantoin and allantoic acid (Table 1), which at least for *C. dewevrei* is in agreement with previous studies (Mazzafera, 1993).

Considering the recovered radioactivity, fruits of *C. dewevrei* seemed to degrade xanthine more efficiently than *C. arabica*. However, it is interesting to observe that the former species proportionally accumulated more xanthine, uric acid, and ureides than *C. arabica*.

Table 2. Concentration of Allantoin and Allantoic Acid in Fruits and Leaves of *C. arabica* and *C. dewevrei*[a]

coffee tissue	allantoin (μg/mg of fresh wt)	allantoic acid (μg/mg of fresh wt)	total (μg/mg of fresh wt)
leaf pair			
C. arabica			
1st	0.195	0.094	0.289
5th	0.262	0.177	0.439
C. dewevrei			
1st	0.199	0.154	0.353
5th	0.186	0.082	0.268
fruits			
C. arabica			
immature	0.406	0.157	0.563
mature	0.418	0.629	1.047
C. dewevrei			
immature	0.582	0.405	0.987
mature	0.479	0.350	0.829

[a] All data are means of two replicates.

Because the recovered radioactivity was determined in the aqueous extract before HPLC analysis, the higher recovered radioactivity in *C. arabica* might be present in compounds formed from ureide degradation. Therefore, the data may indicate that xanthine is more intensively degraded in fruits of *C. arabica* than in *C. dewevrei*. However, leaves of both species had similar values with respect to xanthine degradation (Table 1). In mature fruits of both species higher radioactivity was observed in xanthine and uric acid in mature fruits (Table 1), which indicates that xanthine dehydrogenase and uricase might have lower activities in mature than in immature fruits. On the other hand, except for fruits of *C. arabica*, younger leaves and fruits accumulated more radioactivity in ureides (Table 1), suggesting that the conversion of uric acid to ureides was more effective in these tissues.

Determinations of endogenous allantoin and allantoic acid are shown in Table 2. Considerable amounts of these ureides were found in fruits of both coffee species. In fruits and leaves of *C. dewevrei* there was no clear quantitative distinction, as observed in *C. arabica*, where younger tissues had lower amounts of total ureides. The ureide amounts determined here are, in general, a little higher than those found in soybean seeds, ~0.3 μg/mg (Mosquim and Sodek, 1992), but lower than those determined in comfrey (*Symphytum officinalis*; roots, 7−25.5 μg/mg; leaves, 1 μg/mg), a species known to accumulate allantoin (Sousa et al., 1991).

Table 3 shows the activities of xanthine dehydrogenase, uricase, and urease in leaves and fruits of *C. arabica* and *C. dewevrei*. The observed activities of xanthine dehydrogenase and uricase correlated well with the data of the radioisotope feeding experiments. In leaves, except for xanthine dehydrogenase in the fifth pair of *C. dewevrei* and urease in the fifth pair of *C. arabica*, higher activities were found for both enzymes in young leaves. The same tendency was observed in immature fruits, but, in general, fruits had lower activities than leaves. This could explain why in fruits radioactivity was detected only in xanthine and uric acid.

The activity of xanthine dehydrogenase was tested with four protein amounts from 100 to 1 000 μg, and for uricase it ranged from 1 to 10 μg. The data presented in Table 3 are those obtained in the linear portion of the curve, which were determined in the ranges of 100−

Table 3. Activity of Xanthine Dehydrogenase (XDH), Uricase, and Urease in Fruits and Leaves of *C. arabica* and *C. dewevrei*[a]

coffee tissue	XDH	uricase	urease
leaf pair			
C. arabica			
1st	399	5300	1.25
5th	32.7	1530	2.70
C. dewevrei			
1st	18.3	4180	1.70
5th	25.8	2020	0.75
fruits			
C. arabica			
immature	22.9	3,720	3.25
mature	ND[b]	910	1.00
C. dewevrei			
immature	1.1	580	0.70
mature	ND	350	ND
soybean			
leaves			11.8
cotyledons			513

[a] Activities of XDH and uricase are expressed as ng of uric acid/mg of protein·h and ng of allantoin/mg of protein·h, respectively. The activity of urease in fruits and leaves is given as ng of urea/mg of protein·h and ng of urea/leaf disk·h, respectively. Data are means of two replicates. [b] ND, radioactivity not detectable.

200 and 1−5 μg for xanthine dehydrogenase and uricase, respectively. In both cases the reaction procedures previously described in the literature (see Materials and Methods) were used. On the other hand, the allantoinase and allantoate amidohydrolase activities were not detected using procedures adopted for soybean tissues. In addition, even using different buffers and pH values, extracting with the buffer at the reaction pH, and using protease inhibitors and higher protein and substrate concentrations, we were not able to detect these enzymes.

To test the extraction and assay conditions used for coffee, the activity of allantoinase in protein extracts from soybean leaves was determined and conversions of 7.6 and 15.8% of allantoin in allantoic acid, using 150 and 300 μg of protein in the reaction mixture, respectively, were obtained.

Except for the first leaf pair of *C. arabica*, higher activities of urease were detected in the younger tissues (Table 3). The detection of urease in fruits was only possible using large amounts of protein in the enzymatic assays (1.5−2 mg). Applying the same extraction and assay conditions used for coffee fruits, but, with much less protein (200−300 μg), we were able to detect good urease activity in leaves and cotyledons of germinating soybean seeds, suggesting that our procedures were adequate (Table 3).

DISCUSSION

The enzymes of xanthine metabolism have been more intensively studied in nodulated soybeans, because ureides, which are responsible for most of the transport of fixed nitrogen to the shoots, are formed in the nodules by catabolic degradation of purines synthesized de novo (Schubert and Boland, 1990).

It has been suggested that xanthine is also a product of caffeine degradation in coffee and tea (Suzuki et al., 1992). Several investigations with *C. arabica* showed that labeled xanthine is converted to uric acid, followed by allantoin, allantoic acid, urea + glyoxylic acid, CO_2, and ammonium (Suzuki and Takahashi, 1975; Suzuki and Waller, 1984a,b; Ashihara et al., 1996). As an alternative pathway, it has been shown recently that small portions of xanthine can be methylated by a salvage pathway, forming monomethylxanthines (3- or 7-methylxanthine), which are presumably reutilized for caffeine biosynthesis (Suzuki and Takahashi, 1975; Ashihara et al., 1996, 1997).

In this work, we investigated the main xanthine degradation pathway in two coffee species differing in caffeine catabolism and, for the first time, the activities of the enzymes involved were assayed. Our choice of *C. arabica* and *C. dewevrei* was based on previous investigations that showed that leaves and immature fruits of *C. dewevrei* displayed higher rates for caffeine degradation associated with low biosynthesis, leading to a low caffeine content (Mazzafera et al., 1991, 1994a; Mazzafera, 1993). However, [8-^{3}H]caffeine was used in these studies, and xanthine metabolites could not be investigated.

Our results showing that leaves of both species catabolize xanthine more readily than fruits were substantiated by the enzyme activities determined. In the radioisotope feeding experiments, low radioactivities of xanthine and uric acid were associated with high xanthine dehydrogenase and uricase activities. On the other hand, using procedures already described in the literature, with or without modifications, we were unable to detect allantoinase and allantoate amidohydrolase in both tissues. Similarly, Corpas et al. (1997) could not detect allantoinase activity in peroxisomes purified from pea leaves, although these authors did detect allantoin and xanthine oxidase and uricase activities in these cell organelles.

Therefore, it appears that the levels of allantoinase and allantoate amidohydrolase are very low in coffee tissues, which is consistent with the large amounts of radioactivity found in ureides in the radioisotope feeding experiments and their endogenous levels. However, specific problems of enzymatic inactivation cannot be ruled out. Detection and stability of the enzymes of the caffeine metabolism have been a permanent problem in the studies with this alkaloid. Regarding the caffeine biosynthesis, several studies showed that the *N*-methyltransferases involved are very labile (Roberts and Waller, 1978; Mazzafera et al., 1994b; Kato et al., 1996). Recently, attempts to detect the activity of caffeine demethylase, the first enzyme of caffeine breakdown, were also unsuccessful (Vitória, 1998; Huber and Baumann, 1998).

The physiological role of ureide accumulation in coffee is unknown, but recent data suggest that it may have some importance in N transport in the coffee plant (Mazzafera and Gonçalves, 1999).

One may argue that urease activity bears some relation to ureide accumulation in coffee tissues, as seen in the radioisotope feeding experiments. However, the data presented for ureides in Table 1 and for urease activity in Table 3 in fruits and leaves of *C. dewevrei* and fruits of *C. arabica* would appear to be inconsistent with this idea; that is, low radioactivity in ureides should correlate with high urease activity. However, recently, using urease-negative mutants, Stebbins and Polacco (1995) reported that urease is not essential for ureide degradation in soybean. Therefore, although previous studies have shown labeling of urea in radioisotope feeding experiments, suggesting the involvement of allantoate amidohydrolase, urea formation in coffee

might also be related to arginine degradation (Polacco and Holland, 1993).

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