Plant Physiol. (1970) 46, 103–107

Glutathione Conjugation

ATRAZINE DETOXICATION MECHANISM IN CORN

Received for publication January 9, 1970

R. H. SHIMABUKURO, H. R. SWANSON, AND W. C. WALSH
Metabolism and Radiation Research Laboratory, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Fargo, North Dakota 58102

ABSTRACT

Glutathione conjugation (GS-atrazine) of the herbicide, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) is another major detoxication mechanism in leaf tissue of corn (*Zea mays*, L.). The identification of GS-atrazine is the first example of glutathione conjugation as a biotransformation mechanism of a pesticide in plants. Recovery of atrazine-inhibited photosynthesis was accompanied by a rapid conversion of atrazine to GS-atrazine when the herbicide was introduced directly into leaf tissue. *N*-Dealkylation pathway is relatively inactive in both roots and shoots. The nonenzymatic detoxication of atrazine to hydroxyatrazine is negligible in leaf tissue. The hydroxylation pathway contributed significantly to the total detoxication of atrazine only when the herbicide was introduced into the plant through the roots. The metabolism of atrazine to GS-atrazine may be the primary factor in the resistance of corn to atrazine.

The selective herbicides 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) and 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) are used extensively to control annual weeds in fields of corn and sorghum. These compounds are effective inhibitors of the Hill reaction in photosynthesis (7) and also reduce the rate of $^{14}CO_2$ fixation in plants (1, 16).

The rate of atrazine metabolism in higher plants is an important factor in herbicidal selectivity (9). Detoxication of atrazine was reported to occur by the 2-hydroxylation and *N*-dealkylation pathways in higher plants (9, 10). In sorghum a rapid conversion of atrazine to a water-soluble compound (metabolite B) resulted in a recovery of atrazine-inhibited photosynthesis (14). The subsequent identification of metabolite B as a mixture of two closely related compounds, GS-atrazine[1] and γ-glutamyl-*S*-(4-ethylamino-6-isopropylamino-2-*s*-triazino)cysteine, indicated the presence of a third detoxication pathway present in higher plants (6). In this paper, the mixture will be referred to as GS-atrazine only. A possible precursor-product relationship between the two metabolites was discussed by Lamoureux *et al.* (6).

This investigation was undertaken to determine the significance of the glutathione-atrazine conjugation pathway in corn. Corn is reported to be resistant to atrazine and simazine largely because of its ability to convert the two herbicides to nonphytotoxic hydroxyatrazine and hydroxysimazine (2–4, 8). The hydroxylation reaction is catalyzed nonenzymatically by the cyclic hydroxamate, benzoxazinone, present in corn tissue (2–4, 8). However, an unknown metabolite subsequently identified as GS-atrazine (6) was also detected in the shoots of intact corn plants treated with atrazine through their roots (9).

MATERIALS AND METHODS

Plant Material. Corn seeds (*Zea mays* L. North Dakota KE 47101) were germinated in vermiculite. A group of seedlings were transferred to continuously aerated, half-strength Hoagland's solution after 6 days of germination. Other seedlings were left in vermiculite and intermittently watered with Hoagland's solution. All plants were grown in the greenhouse.

Atrazine Metabolism in Plants. Uniformly ring-labeled atrazine-^{14}C (specific radioactivity 7.8 μc/mg) was purified as previously described (11) and used for treatment of corn. Plants were selected and treated with atrazine in a controlled environment room under conditions described previously (10). Plants grown in nutrient solution were used for root treatment with atrazine-^{14}C. Plants grown in vermiculite were used to study atrazine-^{14}C metabolism in excised leaves and leaf discs. The fourth leaf (30–40 cm) of 24-day-old corn plants was excised under water. Two leaves each were treated by immersing their cut ends into a test tube containing 3 ml of atrazine-^{14}C solution (301,180 dpm). Distilled water was added intermittently to compensate for water loss due to uptake and transpiration. The metabolism of surface-absorbed atrazine-^{14}C was determined by leaf surface application of atrazine-^{14}C in 10% oil in water (v/v) (Sun Superior oil 11 N containing 1% [v/v] Triton X-207) as described previously (14).

Assay for Atrazine-^{14}C and Its Metabolites. Atrazine-^{14}C-treated tissues were extracted with 80% methanol. The methanol in the extract was removed under vacuum to give an aqueous solution. Further purification of the aqueous extract and qualitative and quantitative assay of atrazine-^{14}C and its radioactive metabolites were performed as described previously (9, 11, 12). The ^{14}C activity in chloroform- and water-soluble compounds was determined by liquid scintillation counting (12). Water-soluble radioactive metabolites of atrazine-^{14}C were purified by cation exchange chromatography and separated by TLC for detection and identification as reported (9). Radioactive compounds were determined quantitatively from TLC plates by carefully removing the compounds from the plate and counting by gel scintillation techniques (11).

[1] Abbreviations: GS-atrazine: *S*-(4-ethylamino-6-isopropylamino-2-*s*-triazino)glutathione; hydroxyatrazine: 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine; hydroxysimazine: 2-hydroxy-4,6-bis(ethylamino-*s*-triazine; compound I: 2-chloro-4-amino-6-isopropylamino-*s*-triazine; compound II: 2-chloro-4-amino-6-ethylamino-*s*-triazine; hydroxycompound I: 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine; hydroxycompound II: 2-hydroxy-4-amino-6-ethylamino-*s*-triazine; benzoxazinone: 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine; TLC: thin layer chromatography.

In extracts from corn leaf discs, it was not necessary to wash the aqueous extract with chloroform or to purify the water-soluble metabolites by cation exchange chromatography. Atrazine and its metabolites were readily separated by TLC with minimal interference from water-soluble natural products.

Photosynthesis and Atrazine Metabolism in Leaf Discs. Oxygen evolution was measured in atrazine-treated corn leaf discs to determine photosynthetic inhibition. Leaf discs (8 mm diameter) were cut with a cork borer from the laminae of fully expanded corn leaves. Leaf discs were incubated in atrazine solution for 1.5 hr. Cylindrical 100-ml respirometer flasks were prepared for measuring oxygen evolution on a differential respirometer as previously described (14, 15). Each flask contained 10 leaf discs which were thoroughly rinsed in distilled water at the end of the atrazine incubation period.

Leaf discs were also incubated in atrazine-^{14}C solution for 1.5 hr as above. The discs were thoroughly rinsed as above and placed in respirometer flasks under similar conditions for determination of photosynthetic inhibition and recovery (14). The discs were removed at different time intervals, extracted, and assayed for atrazine-^{14}C metabolism as described previously (9, 11, 12).

RESULTS

Photosynthetic Inhibition and Recovery. Photosynthesis in corn leaf discs recovered after an initial inhibition by atrazine (Fig. 1). Even at the relatively high concentration of 100 μM atrazine, a gradual recovery of photosynthesis was observed within 6 hr. In intermediately susceptible pea, complete inhibition of photosynthesis occurred within 3.5 hr after incubation in 50 μM atrazine (14). However, assay of atrazine-^{14}C metabolism in corn leaf discs did not show the conversion of atrazine-^{14}C to hydroxyatrazine as expected. Atrazine was rapidly converted to GS-atrazine rather than to hydroxyatrazine during the period when rapid recovery of photosynthesis was observed (Fig. 1). A most significant observation was the complete absence of hydroxyatrazine formation during the short period of photosynthetic recovery. The quantitative assay (Table I) indicated that 77% of the atrazine-^{14}C absorbed by the leaf discs was converted to GS-atrazine within 5 hr after the end of the 1.5-hr incubation period. Some radioactivity was found to "leak" out of the discs into the buffer solution present in the flask. An assay of this radioactivity indicated the presence of only unchanged atrazine-^{14}C. This observation may reflect the differences in permeability of the cell membrane to the highly lipophilic atrazine and its hydrophilic metabolite, GS-atrazine.

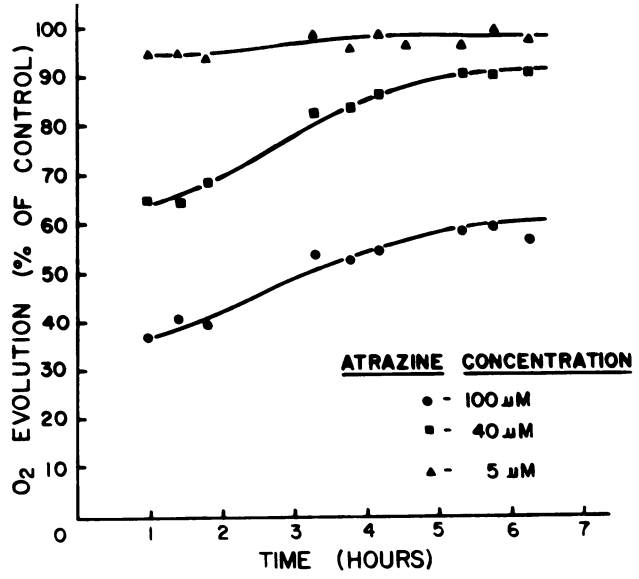

FIG. 1. The inhibition and recovery of oxygen evolution in atrazine-treated corn leaf discs. Oxygen evolution was measured at 25 C, and the respirometer flasks were illuminated from the bottom with a light intensity of about 1400 ft-c. Oxygen evolution rates were recorded at different times over a 6-hr period. Oxygen evolution rates in control ranged from 130 to 150 μl per 10-min interval over the 6-hr period.

Table I. *Atrazine-^{14}C Metabolism in Corn Leaf Discs*

Corn leaf discs were incubated in 55 μM atrazine-^{14}C prepared in 0.5 M phosphate buffer (pH 7.0). Twenty discs per flask were placed under conditions described in Figure 1. The leaf discs in each flask and the ambient buffer were assayed for atrazine-^{14}C and its metabolites at the end of the 1.5-hr incubation period (0 hr) and hourly up to 5 hr. Insoluble residue was determined by dry combustion in oxygen as described (9).

Time	^{14}C Activity Recovered			Distribution of Extracted ^{14}C Activity	
	Extracted	Leakage[1]	Insoluble residue	Atrazine	GS-atrazine
hr	*dpm*			%	%
0	16,700	...	65	81	19
1	11,475	4,320	45	45	55
2	12,200	3,480	25	33	67
3	11,900	2,960	80	32	68
4	13,500	2,320	60	33	67
5	11,475	1,510	70	23	77

[1] This is the ^{14}C activity present in the ambient buffer. Assay of ^{14}C activity indicated the presence of only unchanged atrazine-^{14}C.

The results indicate that in corn, photosynthetic recovery is not necessarily dependent on the detoxication of atrazine by the hydroxylation pathway. This conclusion is contradictory to the previously established concept that the basic factor for resistance in corn is the nonenzymatic hydroxylation of substituted 2-chloro-*s*-triazines to 2-hydroxy-*s*-triazine derivatives (2–4, 8).

Atrazine Metabolism in Excised Leaves and Intact Plants. The predominant atrazine metabolite in corn leaf discs was GS-atrazine (Table I), but a significant concentration of hydroxyatrazine was detected in shoots of intact plants treated with atrazine through their roots (9). These differences may be due to the influence of the roots in intact plants. To approximate normal conditions and yet eliminate the roots as a contributing factor, excised leaves were treated with atrazine-^{14}C for a 30-hr period. Atrazine-^{14}C was absorbed through the cut edge at the base of the leaf and translocated into the leaf blade where metabolism should occur as in an intact plant.

The excised corn leaves absorbed 0.02 μmole of atrazine-^{14}C per gram fresh weight over the 30-hr period. Metabolism of absorbed atrazine-^{14}C during this period occurred very rapidly, with only 1.1% of the total ^{14}C activity remaining as unchanged atrazine. Most of the atrazine was converted to GS-atrazine (82%). Hydroxyatrazine (2.7%), hydroxycompounds I and II (12.2%), and methanol-insoluble residue (2%) accounted for the remaining radioactivity. The results from leaf discs and excised whole leaves indicate that hydroxyatrazine formation in leaf tissue is insignificant over a short period of time and only small amounts are formed over longer periods. The metabolism of atrazine to GS-atrazine seems to be the major detoxication pathway in corn leaf tissue.

Assay of atrazine-^{14}C and its metabolites in the shoots of intact plants treated with atrazine-^{14}C through their roots indicated that high concentrations of both GS-atrazine and

hydroxyatrazine were present in the shoots (Fig. 2). A quantitative assay indicated that within 48 hr, 32% of the ^{14}C activity in the shoots was hydroxyatrazine, 36.5% was GS-atrazine 22.8% was the combined radioactivity in hydroxycompounds I and II, and 8.7% was methanol-insoluble residue. The accumulation of hydroxycompounds I and II at the 144-hr period is readily apparent (Fig. 2). Only trace amounts of hydroxycompound I were present at the 48-hr period. The increased amount of hydroxylated derivatives present in the shoots of intact plants as compared to excised leaves indicate that these metabolites are formed primarily in the roots and translocated to the shoots.

Hydroxyatrazine, absorbed from the leaf surface, was not metabolized to GS-atrazine (Fig. 2). Evidence also indicates that hydroxyatrazine is not formed by the hydrolysis of GS-atrazine in plants. The absence of hydroxyatrazine in sorghum (9), despite the high GS-atrazine concentration in its tissue (6), makes it highly improbable that hydroxyatrazine is derived from the hydrolysis of GS-atrazine. These results indicate that no product-precursor relationship exists between hydroxyatrazine and GS-atrazine.

Metabolism of Surface-Applied Atrazine in Corn Leaves. The results indicate that atrazine is predominantly metabolized to GS-atrazine when the herbicide is introduced directly into leaf tissue, but not when it is absorbed through the roots. Postemergence spraying of an atrazine-oil mixture in corn is becoming a widely used practice. Therefore, metabolism of leaf surface-absorbed atrazine, applied initially as a mixture in oil, was investigated.

The total ^{14}C activity recovered from leaves at the time periods indicated in Table II accounted for 55 to 60% of the total radioactivity initially applied to the leaf surface. No attempt was made to measure the loss of atrazine due to volatilization which has been reported to occur (5). However, translocation studies indicated that no ^{14}C-labeled compounds were translocated out of corn leaves when atrazine-^{14}C was applied to the leaf surface. Ring cleavage and loss of ^{14}C activity as $^{14}CO_2$ did not occur in corn over a 7-day period (9). Therefore, assay of total ^{14}C activity present in leaves would account for all radioactivity initially absorbed as atrazine-^{14}C from the leaf surface.

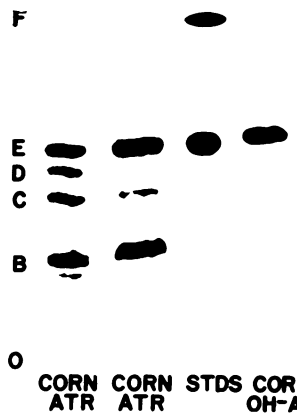

FIG. 2. Metabolism of root-absorbed atrazine-^{14}C and leaf surface-absorbed hydroxyatrazine-^{14}C in corn. Two sets of 3-week-old corn plants were treated by immersing the roots of each set in 400 ml of aerated half-strength Hoagland's solution containing 1.4 μc of atrazine-^{14}C. The plants were placed in a controlled environment room under conditions described previously (10). Only the shoots from each set were harvested after 48 hr (15 g fresh wt) and 144 hr (23 g fresh wt). The shoots were extracted and assayed for atrazine-^{14}C and its metabolites as described in text. Hydroxyatrazine-^{14}C in oil (45,000 dpm) was spotted on corn leaf surface as described previously (14). After 48 hr, leaves were harvested (3 g fresh wt) by rinsing in distilled water, extracted, and assayed for hydroxyatrazine-^{14}C and its metabolites. Left to right: metabolites of atrazine-^{14}C in corn shoots after 144 hr, metabolites of atrazine-^{14}C in corn shoots after 48 hr, authentic atrazine and hydroxyatrazine, metabolite of leaf surface-absorbed hydroxyatrazine-^{14}C. O: Origin; B: GS-atrazine; C: hydroxycompound II; D: hydroxycompound I; E: hydroxyatrazine; F: solvent front (atrazine).

Table II. *Metabolism of Leaf Surface-absorbed Atrazine Applied as Atrazine-Oil Mixture*

One fully expanded leaf of comparable size on separate 3-week-old corn plants was treated by placing droplets of atrazine-^{14}C in 10% oil on the leaf surface. Each leaf was treated with a total volume of 0.2 ml containing 30,000 dpm atrazine-^{14}C. Plants were placed in a controlled environment room under conditions reported previously (10). Representative leaves were harvested at 16, 24, 48, and 144 hr after application. Leaves were rinsed in water, extracted, and assayed for atrazine-^{14}C and its metabolites.

Time	Distribution of ^{14}C Activity			
	Unchanged atrazine	GS-atrazine	Other[1]	Methanol-insoluble residue
hr	%	%	%	%
16	7.9	77.4	13.9	0.8
24	3.9	78.7	15.8	1.6
48	0.4	65.0	27.3	7.2
144	1.9	32.4[2]	28.1	37.6

[1] Includes hydroxyatrazine, hydroxycompounds I and II, and other unidentified compounds.

[2] Includes low concentrations of other water-soluble metabolites with slightly lower R_F values which appear after longer time periods.

The results indicated that surface-absorbed atrazine-^{14}C was rapidly converted to GS-atrazine in the leaf tissues (Table II). Maximum concentrations of the peptide conjugate accumulated within 16 to 24 hr after leaf surface application of the herbicide. Lower concentrations of hydroxyatrazine and hydroxycompounds I and II were detected over a period of 144 hr. The concentration of GS-atrazine decreased by 50% between 48- and 144-hr periods, and the radioactivity in methanol-insoluble residue increased significantly during the same period. The increase in ^{14}C activity present as insoluble residue may be directly related to the decrease in GS-atrazine concentration. The ^{14}C activity in hydroxylated metabolites remained fairly constant between 48- and 144-hr periods. The results suggest that the peptide conjugate may be an intermediate in the incorporation of the *s*-triazine ring into methanol-insoluble residue in corn.

DISCUSSION AND CONCLUSION

The recovery of photosynthetic oxygen evolution with the concomitant appearance of GS-atrazine in corn leaf discs indicates that the metabolism of atrazine to its peptide conjugate is a detoxication mechanism. Apparently, the major detoxication mechanism in corn is not necessarily limited to the nonenzymatic hydroxylation of atrazine to nonphytotoxic hydroxyatrazine. The nonenzymatic hydroxylation of substituted 2-chloro-*s*-triazines has been the accepted explanation for resistance or selectivity in corn (2–4, 8). However, the results indicate that detoxication of atrazine by hydroxylation is negligible when the herbicide is introduced directly into leaf tissue (Tables I, III).

A significant concentration of hydroxyatrazine was present in shoots of corn plants only when atrazine was absorbed through the roots (Fig. 2). The results suggest that the root may be

Table III. *Summary of Atrazine Detoxication Pathways in Selected Plant Species*

Plant	Response to Atrazine	Relative Activity[1]			Reference
		Pathway A[2]	Pathway B[2]	Pathway C[2]	
Corn	Resistant				9, 10
Shoot		+	+	++++	
Root[3]		+	++++	+	
Sorghum	Resistant				6, 9, 14
Shoot		++	−	++++	
Root[3]		++	−	+	
Pea	Intermediate				9, 11, 12
Shoot		++++	−	+	
Root		++++	−	+	
Cotton[4]	Intermediate	++++	−	+	13
Soybean[4]	Susceptible	++	−	+	9
Wheat[4]	Susceptible	+	+	+	9

[1] + indicates the presence and − indicates the absence of the pathway in a particular plant. The activities of the pathways, as indicated by the number of +, are relative, and direct comparison of rates between pathways is not intended.

[2] These pathways are shown in Figure 3.

[3] Unpublished data (Frear and Swanson) indicate that enzyme activity for GS-atrazine formation is extremely low in roots.

[4] Relative activities based on data from whole plant.

primarily responsible for the formation of hydroxyatrazine. It is likely that most of the hydroxyatrazine present in the shoots of corn plants was translocated as the metabolite from the roots.

The rate of atrazine metabolism and detoxication seems to determine the resistance of higher plants to the herbicide (9). The evidence indicates that higher plants are capable of detoxifying atrazine by at least three different metabolic pathways (Fig. 3) (6, 10, 12). Pathway A (*N*-dealkylation) (labeled at the bottom of Fig. 3) is probably enzymatic, while the enzyme responsible for glutathione conjugation (Pathway C) has been isolated and identified in corn (Frear and Swanson, unpublished data). Pathway B involves both nonenzymatic (benzoxazinone-catalyzed hydrolysis) and enzymatic (*N*-dealkylation) reactions to give several nonphytotoxic products (10). The relative activities of the three atrazine detoxication pathways in selected plant species are presented in Table III. Pathway B seems to be limited to species such as corn and wheat which contain benzoxazinone (9). Pathway C appears to be very active in the highly resistant species, corn and sorghum (6, 14). Pathway A was shown to be extremely important in intermediately susceptible species such as pea (9, 11) and cotton (13) where pathway B is nonexistent and pathway C is relatively inactive.

All three pathways are known to exist in corn. However, pathway A is relatively inactive (9), while pathways B and C are extremely active. This investigation indicated that in corn the rate of atrazine detoxication by pathway B or C was dependent on the path of entry into the plant. Atrazine was metabolized primarily through pathway C when the herbicide was introduced directly into the leaves. Pathways B and C contributed significantly to the detoxication of atrazine when the herbicide was absorbed through the roots.

The results suggest that the nonenzymatic hydrolysis of atrazine to hydroxyatrazine may not be the basic factor for selectivity in corn. The presence of benzoxazinone in corn invariably contributes to total atrazine detoxication, as demonstrated in root-fed plants. However, in the leaves of corn where the atrazine-sensitive sites (*i.e.*, chloroplasts) are located, recovery of atrazine-inhibited photosynthesis occurred without the participation of an active hydroxylation pathway. The basic factor for herbicidal selectivity in intact corn plants may become evident if herbicidal resistance is determined in the presence of pathways B and C, and in the absence of one of the major pathways. Further studies are now in progress to show whether the enzymatic detoxication of atrazine to GS-atrazine is the basic factor responsible for atrazine selectivity in corn.

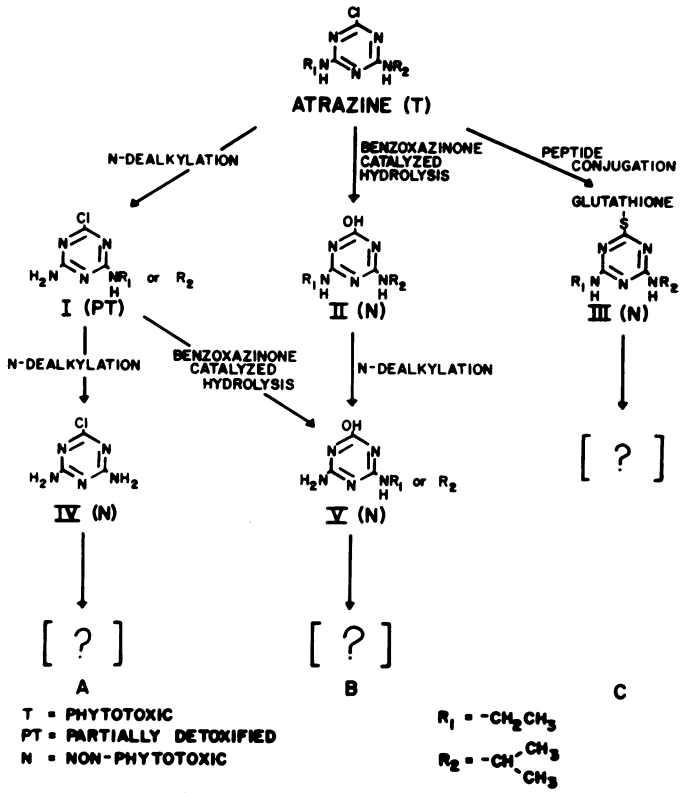

FIG. 3. Metabolic scheme for atrazine metabolism in higher plants. I: Compounds I and II; II: hydroxyatrazine; III: GS-atrazine; IV: 2-chloro-4,6-diamino-*s*-triazine (isolated from sorghum and identified by infrared and mass spectrometry, unpublished data); V: hydroxy-compounds I and II.

Acknowledgment—Radiocarbon-labeled and nonlabeled atrazine and hydroxyatrazine were generously provided by Geigy Chemical Corporation, Ardsley, New York.

LITERATURE CITED

1. ASHTON, F. M., G. ZWEIG, AND G. MASON. 1960. The effect of certain triazines on $^{14}CO_2$ fixation in red kidney beans. Weeds 8: 448–451.
2. CASTELFRANCO, P., C. L. FOY, AND D. B. DEUTSCH. 1961. Non-enzymatic detoxification of 2-chloro-4,6-*bis*(ethylamino)-*s*-triazine (simazine) by extracts of *Zea mays*. Weeds 9: 580–591.
3. GYSIN, A. AND E. KNÜSLI. 1960. Chemistry and herbicidal properties of triazine derivatives. Advan. Pest Contr. Res. 3: 289–358.
4. HAMILTON, R. H. AND D. E. MORELAND. 1962. Simazine degradation by corn seedlings. Science 135: 373–374.
5. KEARNEY, P. C., T. J. SHEETS, AND J. W. SMITH. 1964. Volatility of seven *s*-triazines. Weeds 12: 83–87.
6. LAMOUREUX, G. L., R. H. SHIMABUKURO, H. R. SWANSON, AND D. S. FREAR. 1970. The metabolism of 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) in excised sorghum leaf sections. J. Agr. Food Chem. 18: 81–86.
7. MORELAND, D. E., W. A. GENTNER, J. L. HILTON, AND K. L. HILL. 1959. Studies on the mechanism of herbicidal action of 2-chloro-4,6-bis (ethylamino)-*s*-triazine. Plant Physiol. 34: 432–435.
8. ROTH, W. 1957. Etude comparée de la réaction due mäis et du blé à la simazine, substance herbicide. Compt. Rend. 245: 942–944.
9. SHIMABUKURO, R. H. 1967. Atrazine metabolism and herbicidal selectivity. Plant Physiol. 42: 1269–1276.
10. SHIMABUKURO, R. H. 1968. Atrazine metabolism in resistant corn and sorhgum. Plant Physiol. 43: 1925–1930.
11. SHIMABUKURO, R. H. 1967. The significance of atrazine dealkylation in root and shoot of pea plants. J. Agr. Food Chem. 15: 557–562.
12. SHIMABUKURO, R. H., R. E. KADUNCE, AND D. S. FREAR. 1966. Dealkylation of atrazine in mature pea plants. J. Agr. Food Chem. 14: 392–395.
13. SHIMABUKURO, R. H. AND H. R. SWANSON. 1970. Atrazine metabolism in cotton as a basis for intermediate tolerance. Weed Sci. 18: 231–234.
14. SHIMABUKURO, R. H. AND H. R. SWANSON. 1969. Atrazine metabolism, selectivity, and mode of action. J. Agr. Food Chem. 17: 199–205.
15. SWANSON, C. R. AND H. R. SWANSON. 1968. Metabolic fate of monuron and diuron in isolated leaf discs. Weed Sci. 16: 137–143.
16. ZWEIG, G. AND F. M. ASHTON. 1962. The effect of 2-chloro-4-ethylamino-6-iso propylamino-*s*-triazine (atrazine) on distribution of ^{14}C compounds following $^{14}CO_2$ fixation in excised kidney bean leaves. J. Exp. Bot. 13: 5–11.