Atrazine Metabolism, Selectivity, and Mode of Action

Richard H. Shimabukuro and Harley R. Swanson

The primary atrazine-sensitive site seems to be located within the chloroplast for resistant as well as susceptible plants. Atrazine inhibits the Hill reaction and its associated noncyclic photophosphorylation, while being ineffective against cyclic photophosphorylation. *N*-Dealkylation causes a decrease in the inhibitory activity of atrazine, but the same reactions mentioned above were inhibited despite the change in molecular structure. Atrazine readily penetrates the chloroplasts of resistant as well as susceptible plants and seems to accumulate there until an equilibrium concentration is attained between the chloroplasts and the cytoplasm. In resistant plants such as sorghum, metabolism of atrazine very likely occurs outside the chloroplasts to form water-soluble compounds and insoluble residue, reduces the concentration of the photosynthetic inhibitor in the chloroplasts, and results in a recovery of photosynthesis. Changes in solubility of the parent atrazine, caused by its metabolism, may be of great significance in the tolerance of plants to the herbicide.

The chemical and biological properties of substituted *s*-triazines have been discussed by Gysin and Knüsli (1960). Two substituted *s*-chlorotriazines widely used as selective herbicides in fields of corn and sorghum, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) and 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine), seem to affect higher plants in a similar manner. Their high biological activity against a wide spectrum of plants has made atrazine and simazine useful as selective herbicides.

Selectivity seems to depend on the extent to which a herbicide remains in its toxic form in the plant. Therefore, the persistence and effectiveness of kill may be directly influenced by the metabolism and detoxication of the herbicide in an intact plant. Metabolism and detoxication of atrazine occur in two possible ways in higher plants (Shimabukuro, 1967a). Dechlorination of atrazine and simazine in corn by the conversion of the *s*-chlorotriazines to their 2-hydroxy derivatives (hydroxyatrazine and hydroxysimazine) is an important detoxication mechanism (Castelfranco *et al.*, 1961; Gysin and Knüsli, 1960; Hamilton and Moreland, 1962; Roth, 1957). This reaction seems to be catalyzed nonenzymatically by a cyclic hydroxamate, 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine (benzoxazinone) (Castelfranco *et al.*, 1961; Hamilton and Moreland, 1962; Roth, 1957; Roth and Knüsli, 1961). *N*-Dealkylation of atrazine, which forms partially detoxified 2-chloro-4-amino-6-isopropylamino-*s*-triazine (compound I) and 2-chloro-4-amino-6-ethylamino-*s*-triazine (compound II) (Shimabukuro *et al.*, 1966; Shimabukuro, 1967a, b), is another detoxication mechanism present in higher plants. All higher plants probably metabolize atrazine by *N*-dealkylation to some extent, while species such as corn and wheat, which contain benzoxazinone, utilize hydroxylation as well (Shimabukuro, 1967a). The ability to metabolize atrazine may not necessarily render a plant resistant to atrazine, since resistant, intermediately susceptible, and susceptible species all seem capable of detoxifying atrazine at different rates.

The principal mode of action of atrazine seems to be the inhibition of photosynthesis in higher plants (Ashton *et al.*, 1960; Gast, 1958; van Oorschot and Belksma, 1961). Exer (1961, 1958) and Moreland *et al.* (1959) reported the inhibition of the Hill reaction by simazine. Good (1961) and Bishop (1962, 1958) showed that triazines such as atrazine acted in a similar manner as 3-(3,4-dichlorophenyl)-1.1-dimethylurea (diuron) by inhibiting the mechanism involved in oxygen evolution. Ashton *et al.* (1963a, b) reported that atrazine caused morphological and cytological changes in bean leaves. However, it was postulated that these changes were brought about only after an unknown interaction had occurred between atrazine, light, and chlorophyll. Results of the different investigations on the mode of action of triazine herbicides definitely indicate that the primary atrazine-sensitive site in higher plants is located in the chloroplast.

Hill reaction in isolated chloroplasts from resistant and susceptible plants were equally inhibited by simazine (Moreland and Hill, 1962). If metabolism of atrazine is the basis for selectivity, what is its relationship with regard to the atrazine-sensitive site in resistant and susceptible species? Is the difference in phytotoxicity between atrazine and its metabolic derivatives (Shimabukuro, 1967a) due to changes in molecular structure? Does metabolism alter physical properties, such as solubility, which prevent penetration of the active molecule to the sensitive site in the chloroplasts, as suggested by Good (1961)?

MATERIALS AND METHODS

Plant Material. Pea seeds, *Pisum sativum* (L.) variety Little Marvel, and sorghum seeds, *Sorghum vulgare* (Pers.) variety North Dakota 104, were germinated and grown in vermiculite. Plants were watered intermittently with one-half strength Hoagland's nutrient solution. When not otherwise specified, pea and sorghum plants were grown in the greenhouse.

Assay for Atrazine-^{14}C and Its Metabolites. The absorption and metabolism of atrazine in pea and sorghum

Metabolism and Radiation Research Laboratory, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Fargo, N. D.

leaf tissue were determined by treating the plant material with uniformly ring-labeled atrazine-^{14}C (specific activity 7.8 μc. per mg.), purified prior to use as reported (Shimabukuro, 1967b). Extraction of plant tissues, purification of the extract, and qualitative and quantitative assay of atrazine-^{14}C and its radioactive metabolites were performed as described previously (Shimabukuro *et al.*, 1966; Shimabukuro, 1967a, b).

Photosynthesis and Atrazine Metabolism in Leaf Disks. Oxygen evolution was measured to determine photosynthetic rates in sorghum and pea leaf disks. Preparation of leaf disks for measuring photosynthetic rates was similar to the procedure described by Swanson and Swanson (1968a).

Leaf disks, 8-mm. diameter, were cut from fully expanded leaves of plants growing in the greenhouse. These plants had 2 hours of daylight illumination prior to the experiment. Pea plants were 1 month old, while sorghum plants were from 1.5 to 2 months old. Leaf disks were incubated in atrazine solution for 1.5 hours and transferred to cylindrical 100-ml. respirometer flasks. Ten disks in each of three flasks were placed in 0.05*M* phosphate buffer (pH 7.0) as described by Swanson and Swanson (1968a). The center well contained 5.0 ml. of CO_2 buffer (Pardee, 1949), which maintained an atmosphere of 0.3% CO_2 in the flask at 25° C. The flasks were placed in the water bath (25° C.) of a Gilson differential respirometer and illuminated with *ca.* 1400 foot-candles for 1 hour before oxygen evolution rates were recorded at 15- to 30-minute intervals over the next 6- to 7-hour period.

Atrazine metabolism in leaf disks was determined by incubating 15 sorghum or pea leaf disks in 10 ml. of $5 \times 10^{-5}M$ atrazine-^{14}C (1.9×10^6 d.p.m.) solution prepared in phosphate buffer. After a 1.5-hour incubation period, the leaf disks were thoroughly rinsed, placed in respirometer flasks, and maintained under conditions described above. At the end of 7 hours, the total ^{14}C activity in the ambient buffer was measured, and the labeled compounds present were identified. The leaf disks were extracted, and the ^{14}C activity, present as chloroform- and water-soluble compounds and methanol-insoluble plant residue, was determined.

Chloroplast Isolation and Photochemical Activity. Freshly harvested pea leaves were used for whole chloroplast isolation according to a procedure described by Whatley and Arnon (1962). Approximately 10 grams of leaf material were ground in 30 to 40 ml. of 0.35*M* NaCl–0.2*M* Tris buffer (pH 8.0) mixture. Final volume of suspended chloroplasts in 0.35*M* NaCl was 5 ml. Chlorophyll concentration was determined by the method of Arnon (1949).

Cyclic photophosphorylation was measured according to the method of Whatley and Arnon (1962). The reaction mixture consisted of Tris-HCl buffer, pH 8.0 (80 μmoles), $MgCl_2$ (10 μmoles), adenosine diphosphate, pH 8.0 (10 μmoles), phenazine methosulfate (PMS) (0.1 μmole), K_2HPO_4 (10 μmoles), and 0.2 ml. of chloroplast preparation containing 100 to 150 μg. of chlorophyll, which was added from the side arm of a manometer vessel. Aqueous solutions of the inhibitors were added to obtain the desired concentration in a final reaction volume of 3.0 ml. The vessels were attached to the manometers of the Gilson differential respirometer, placed in the water bath at 15° C., and flushed with nitrogen for 5 minutes before starting the reaction. The reaction was started by adding the chloroplast preparation from the side arm into the vessel and turning on the lights (1400 foot-candles). The reaction was terminated after 15 to 20 minutes by turning off the lights and adding 0.3 ml. of 20% trichloroacetic acid to the reaction mixture. The esterification of inorganic phosphate was followed by analysis of the acidified reaction mixture for inorganic phosphate, using the molybdenum blue method of Fiske and Subarrow (1925).

The procedure for measuring noncyclic photophosphorylation was similar to that for cyclic photophosphorylation, except for the substitution of $K_3Fe(CN)_6$ (10 μmoles) as the electron acceptor in place of PMS in the reaction mixture. The Hill reaction activity was also measured at the end of the reaction period by determining absorbance changes at 420 mμ due to ferricyanide reduction.

Penetration and Accumulation of Atrazine-^{14}C and Its Metabolites in Chloroplasts. This experiment was performed to determine the concentration of atrazine-^{14}C and its metabolites in the chloroplasts of intact leaf tissue after absorption of atrazine-^{14}C from the leaf surface. Greenhouse-grown 1-month-old pea and 2-month-old sorghum plants were placed in a controlled environment room under conditions previously reported (Shimabukuro, 1967b) for 24 hours before treatment.

A solution of the uniformly ring-labeled atrazine-^{14}C in 10% oil (Sun Superior oil 11N containing 1% Triton X-207) was used for leaf surface application. All the leaves on each pea plant and six of the uniformly mature middle leaves on each sorghum plant were treated by spotting the atrazine-^{14}C solution on the upper leaf surface with a microsyringe. A total of 1.2 ml. of the atrazine-^{14}C solution (180,000 d.p.m.) was applied to each sorghum plant and to three pea plants. The treated leaves from one sorghum and three pea plants (approximately 10 grams of leaf tissue for each species) were harvested after 16, 72, and 168 hours from time of treatment for chloroplast isolation. The leaves were rinsed in water, and the chloroplasts were isolated. Radioactivity, present as chloroform-soluble and water-soluble compounds in the chloroplast pellet and the supernatant, was assayed.

A sample of pea and sorghum leaf tissues, harvested at the 168-hour period, was exhaustively extracted with methanol, and the total soluble and insoluble ^{14}C activity present in the leaves was determined. This was necessary to ascertain what part of the total ^{14}C activity present in the leaves at the same time period was extracted by the chloroplast isolation procedure. Due to the nature of the chloroplast isolation procedure, some of the soluble ^{14}C activity and all of the insoluble ^{14}C activity present in the discarded sand and leaf debris were not accounted for. The recovery of ^{14}C activity is discussed in the results section.

RESULTS

Inhibition of Hill Reaction and Photophosphorylation. The inhibition of the Hill reaction in isolated pea chloroplasts gave half maximal inhibitory concentrations (I_{50}) of $2.0 \times 10^{-6}M$ for atrazine and $4.6 \times 10^{-5}M$ for compound I. The activity of compound II was similar to compound I. Therefore, the I_{50} for compound I is 23 times greater than the I_{50} for atrazine. The results of the Hill reaction inhibition experiments agreed with the reported bioassay results with susceptible oat (Shimabukuro, 1967a) and intermediately susceptible pea (Shimabukuro, 1967b). In these bioassays, atrazine was the phytotoxic form of the herbicide. *N*-Dealkylation of atrazine led to partial detoxication of the herbicide. Metabolism of atrazine to more highly polar derivatives very likely led to complete detoxication (Shimabukuro, 1967a).

The results in Table I indicate that atrazine and its biologically active derivative, compound I, act in a similar manner as diuron on the photosynthetic system. Diuron is a powerful inhibitor of the Hill reaction and inhibits noncyclic photophosphorylation, while having little effect on cyclic photophosphorylation (Jagendorf and Avron, 1959). At $10^{-7}M$ concentration, atrazine inhibited ferricyanide-dependent, noncyclic photophosphorylation by *ca.* 30%, as compared to *ca.* 80% for diuron. PMS-dependent cyclic photophosphorylation was insensitive to diuron, atrazine, and compound I. Only diuron, at a very high concentration of $10^{-4}M$, showed any effect. The results on compound I show that *N*-dealkylation definitely reduced the activity of atrazine, but very likely did not change the mode of action. The data suggest that atrazine and its active monodealkylated derivatives have the same principal mode of action—i.e., the inhibition of the Hill reaction in photosynthesis.

Photosynthetic Inhibition and Recovery. The ability of resistant plants to metabolize and detoxify atrazine rapidly is the basis for selectivity in higher plants (Shimabukuro, 1967a). If the principal mode of action of atrazine is the inhibition of the Hill reaction, a positive correlation between the rates of photosynthesis and metabolism of atrazine should exist in leaf tissue.

Table I. Photophosphorylation in Isolated Pea Chloroplasts

Compound	Photophosphorylation (% of Control) Dependent on[a] PMS	Ferricyanide
Control	100.0	100.0
Diuron ($10^{-7}M$)	96.3	21.1
Diuron ($10^{-6}M$)	107.0	0.0
Diuron ($10^{-4}M$)	84.5	0.0
Atrazine ($10^{-7}M$)	100.0	71.2
Atrazine ($10^{-6}M$)	100.0	0.0
Atrazine ($10^{-5}M$)	92.0	0.0
Compound I ($10^{-6}M$)	93.0	63.4
Compound I ($10^{-5}M$)	93.0	0.0

[a] Results based on μmoles ATP formed per mg. of chlorophyll per hour. PMS control–221, ferricyanide control–139.

Van Oorschot (1965) demonstrated the recovery of CO_2 uptake after an initial inhibition in resistant corn plants treated with simazine via the roots. Although metabolism of simazine was not determined, there is reason to believe that metabolism was a factor in photosynthetic recovery.

If the primary atrazine-sensitive site is located in the chloroplast, experiments using leaf disks may simulate what occurs in leaves of intact plants. The inhibition and recovery of photosynthesis in leaf disks of sorghum and pea (Figure 1) agreed with the reported tolerances of intact sorghum and pea plants to atrazine. Both species were reported to be capable of metabolizing atrazine, but at different rates (Shimabukuro, 1967a). Figure 1 indicates that over a relatively short 7-hour period, an increasing inhibition of photosynthesis was observed in pea. But in sorghum, an apparent recovery occurred with time. In each species, the degree of inhibition was greater at $5.0 \times 10^{-5}M$ atrazine than $1.0 \times 10^{-5}M$. In pea, complete inhibition occurred within 3.5 hours after incubation in $5.0 \times 10^{-5}M$ atrazine.

An analysis of atrazine metabolism over the 7-hour period indicated that sorghum rapidly formed a highly polar derivative called metabolite B (Figure 2, Table II). This water-soluble derivative (R_f 0.29) was also reportedly the predominant water-soluble metabolite found in shoots of intact sorghum plants within 48 hours (Shimabukuro, 1967a). The *N*-dealkylated derivatives, compounds I and II, were not detected in leaf disks of either sorghum or pea within a 7-hour period. Table II and Figure 2 indicate that no detectable metabolism of atrazine occurred in pea during the same period.

Metabolite B is more polar than the nonphytotoxic derivatives of atrazine, hydroxyatrazine, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (hydroxy compound I), and 2-hydroxy-4-amino-6-ethylamino-*s*-triazine (hydroxy compound II), reported in resistant corn (Shimabukuro, 1967a, 1968). The increased polarity of metabolite B, as compared to the parent atrazine, may be the basis for its inactivation.

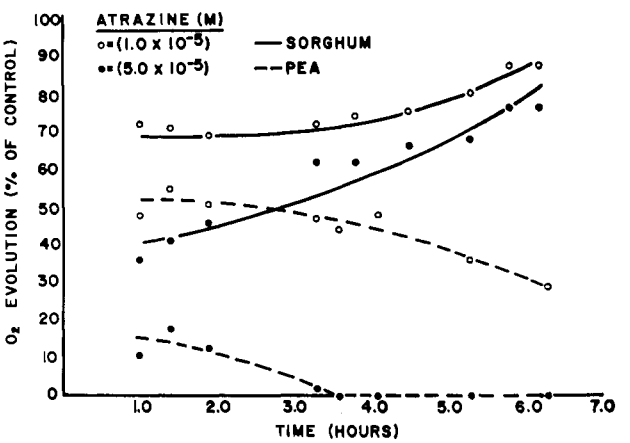

Figure 1. Inhibition and recovery of oxygen evolution in sorghum and pea leaf disks after a 1.5-hour incubation period in atrazine solution

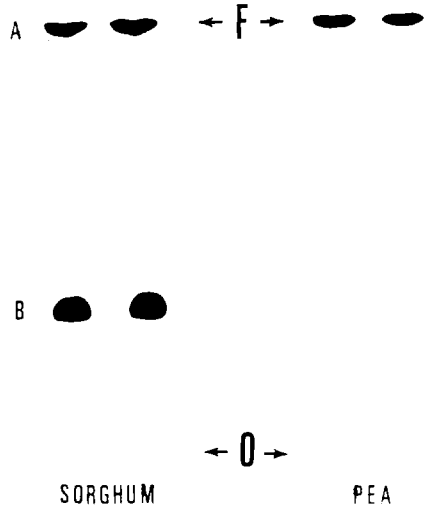

Figure 2. Autoradiogram of thin-layer chromatogram developed by multiple chromatography (Shimabukuro, 1967a)

Two spots each from sorghum (left) and pea (right) leaf disk extracts are shown. O—origin, F—front, A—unchanged atrazine, B—water-soluble metabolite B (R_f 0.29)

Table II. Atrazine Metabolism in Sorghum and Pea Leaf Disks

	Distribution of Total ^{14}C Activity, %			
	0-Hour Period [a]		7-Hour Period [b]	
Compound	Sorghum	Pea	Sorghum	Pea
Metabolite B	14.5	—	62.3	—
Atrazine	77.2	100.0	21.9	100.0
Unidentified	1.8	—	2.6	—
Insoluble residue	6.5	—	13.2	—

[a] Results of atrazine metabolism in the leaf disks at the end of the 1.5-hour incubation period. Leaf disks were rinsed thoroughly at the end of this period before they were placed in buffer solution.

[b] Figures represent distribution of ^{14}C activity present in the leaf tissue. Of the total radioactivity recovered [^{14}C activity in leaf tissue and buffer solution ("leakage")], 39.2 and 50% were present as "leakage" of sorghum and pea leaf disks, respectively.

Table II indicates that after 7 hours most of the radioactivity present in sorghum leaf disks was metabolite B. In pea only unchanged atrazine was detected. Some radioactivity was found to "leak" out of the leaf disks into the surrounding buffer. An analysis of the "leakage" indicated the presence of only unchanged atrazine in both sorghum and pea samples. The absence of metabolite B in the "leakage" of sorghum may reflect differences in permeability to biological membranes between the highly lipophilic atrazine and the hydrophilic metabolite B.

The results indicate that photosynthesis is inhibited by atrazine in both sorghum and pea, but a recovery of photosynthesis in a resistant species can occur very readily. In sorghum the recovery seems to be closely associated with the conversion of atrazine to a highly water-soluble derivative.

Penetration and Accumulation of Atrazine-^{14}C and Its Metabolites in Chloroplasts. The exact mechanism by which atrazine produces its inhibition is still unknown. However, atrazine must penetrate the chloroplasts of sorghum and pea and reach the reactive site involved in the oxidation of water. If the degree of inhibition of the Hill reaction is equal to the number of reactive sites bound by the inhibitor, as reported (Izawa and Good, 1965), the inhibition produced should then be related to the concentration of atrazine in the chloroplast. Moreland and Hill (1962) demonstrated that isolated chloroplasts from tolerant and susceptible species were equally sensitive to simazine and other Hill reaction inhibitors. Therefore, the chloroplast itself may not be involved in selectivity. The results presented in this investigation indicate that metabolism of atrazine is related to the recovery of Hill reaction activity in resistant sorghum leaf disks. Therefore, a relationship must exist between the metabolism of atrazine, the concentration of the inhibitor in the chloroplasts, and the recovery of photosynthesis from its inhibited rate.

Figure 3 shows the relationship between metabolism of atrazine and its concentration in the chloroplasts over a period of 168 hours. Because of the procedure used to obtain these results, the data represent only the soluble ^{14}C activity extracted from intact leaves at the three time periods. Determination of atrazine-^{14}C absorption indicated that within 24 hours only 10 to 15% of the applied atrazine-^{14}C remained on the leaf surface. No attempt was made to measure the atrazine-^{14}C loss due to volatilization which has been reported to occur (Kearney *et al.*, 1964). Between 75 and 80% of the applied ^{14}C activity was present in the leaf tissue. Translocation studies indicated that no ^{14}C-labeled compounds were translocated out of sorghum and pea leaves. The loss of ^{14}C activity as $^{14}CO_2$ over a 7-day period in uniformly ring-labeled atrazine-^{14}C-treated sorghum plants was reported to be negligible (Shimabukuro, 1967a). Therefore, metabolism and distribution of atrazine-^{14}C occurring in the treated leaves may be considered as changes occurring

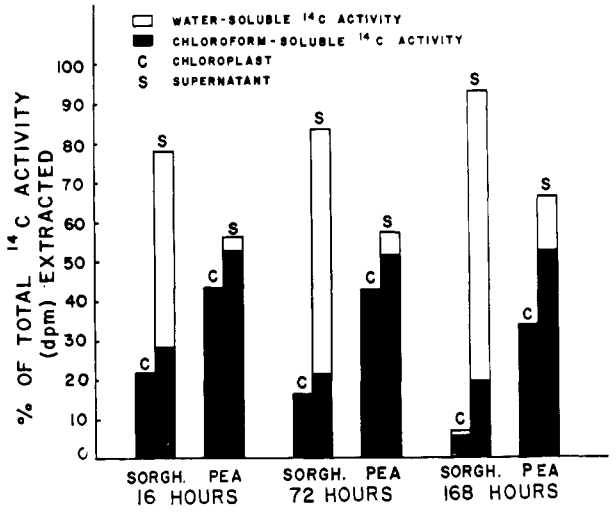

Figure 3. Distribution of chloroform-soluble and water-soluble ^{14}C activity between the chloroplasts and supernatant of intact sorghum and pea leaves

in an isolated system, as far as the fate of surface-absorbed atrazine-^{14}C is concerned.

Chlorophyll concentration in isolated chloroplasts gave the following values for 16-, 72-, and 168-hour periods, respectively: pea, 1.12, 1.31, and 1.16 mg. per ml.; sorghum, 0.78, 0.73, 0.75 mg. per ml. The quantitative analysis was based on a total volume of 5 ml. prepared from the chloroplast pellet isolated from 10 grams of atrazine-^{14}C-treated leaf material. If chlorophyll concentration in the chloroplasts does not change significantly due to atrazine treatment, nearly equal numbers of chloroplasts at three time periods were probably involved in the partitioning of the chloroform-soluble compounds between chloroplasts and the remainder of the cell (hereafter referred to as the supernatant or cytoplasm) in each species.

To determine how much of the total ^{14}C activity in the leaf tissue was extracted by the chloroplast isolation procedure, a comparison was made between leaves extracted by the chloroplast isolation procedure and by the exhaustive extraction procedure. Separate 168-hour leaf samples of sorghum and pea were extracted by the two methods for comparison. The results indicated that the soluble ^{14}C activity extracted by the chloroplast isolation procedure accounted for 75 to 85% of the methanol-soluble ^{14}C activity extracted by the exhaustive procedure in pea and sorghum. Therefore, one should be aware that the results plotted in Figure 3 represent only the ^{14}C activity extracted and not the total ^{14}C activity present in the tissues.

The difference between sorghum and pea is much greater than shown in Figure 3 if the total ^{14}C activity in the leaf tissue is considered. Exhaustive extraction results indicated that at 168 hours, 54% of the radioactivity in sorghum was methanol-insoluble, while in pea, only 3% was methanol-insoluble. Therefore, the methanol-soluble ^{14}C activity used in plotting Figure 3 represents a smaller portion of the total radioactivity in sorghum than in pea. These results reflect the difference in rate of atrazine metabolism between sorghum and pea (Shimabukuro, 1967a).

Figure 3 indicates that the concentration of chloroform-soluble ^{14}C activity in the chloroplasts was only slightly less than the concentration found in the supernatant at each time period in both sorghum and pea. However, the ^{14}C activity present in the chloroplasts decreased, while the activity in the supernatant showed a relative increase for both species over the 168-hour period. This increase of ^{14}C activity in the supernatant in relation to the activity in the chloroplasts was more pronounced in sorghum than pea. The relative increase of radioactivity in the supernatant was due primarily to an increase in the water-soluble ^{14}C activity. This was especially true in sorghum, where 79% of the ^{14}C activity present in the supernatant at the 168-hour period was water-soluble. Only a small amount of the water-soluble ^{14}C activity was found in sorghum chloroplasts at the same period. Apparently, the more polar, water-soluble metabolite(s) of atrazine-^{14}C present in the supernatant does not penetrate and accumulate in the chloroplasts of sorghum and pea. This is in contrast to the chloroform-soluble compounds.

The chloroform-soluble ^{14}C activity in the chloroplasts and supernatant of sorghum was predominantly unchanged atrazine. Only a trace of compound I appeared at the 168-hour period. In pea, *N*-dealkylation occurred quite readily and the accumulation of compound I was observed in both the chloroplasts and supernatant (Table III). At any of the three time periods, the relative concentrations of unchanged atrazine to compound I in the chloroform-soluble fractions of pea chloroplasts and supernatant were the same (Table III). It seems that atrazine and its highly lipophilic derivative, compound I, readily penetrate the chloroplasts and reach an apparent equilibrium concentration between the chloroplasts and supernatant. The data indicate that the equilibrium concentrations of atrazine and compound I were dependent on the rate of atrazine metabolism by *N*-dealkylation.

The predominant compound in the water-soluble fractions of both sorghum and pea supernatant was metabolite B, also found in sorghum leaf disks (Figure 2). In sorghum, a second more polar metabolite with an R_f value of 0.24 (metabolite B—R_f 0.29) (developed by multiple chromatography) appeared at the 72-hour and 168-hour periods. This agreed with results reported for intact plants treated with atrazine via the roots (Shimabukuro, 1967a).

DISCUSSION AND CONCLUSIONS

The recovery of photosynthesis with the concomitant metabolism of diuron in leaf disks was demonstrated in cotton by Swanson and Swanson (1968a, b). A similar recovery of photosynthesis in sorghum with the concomitant metabolism of atrazine clearly implicated metabolism as an inactivating process. Metabolic inactivation of atrazine restored the photochemical activity in illuminated leaf disks of resistant sorghum plants.

The data presented agree with the hypothesis that

Table III. Chloroform-Soluble Radioactive Compounds in the Chloroplast and Supernatant of Pea Leaf Tissue

	Distribution of ^{14}C Activity, %					
	16-Hour		72-Hour		168-Hour	
	Atrazine	Compound I[a]	Atrazine	Compound I	Atrazine	Compound I
Chloroplast pellet	87	13	61	39	39	61
Supernatant	86	14	68	32	38	62

[a] Compound I was the predominant *N*-dealkylated derivative of atrazine present in pea. Compound II was present only in trace amounts.

the inhibition of the Hill reaction is the primary photosynthetic process affected by atrazine. Ferricyanide reduction and the associated ferricyanide-dependent photophosphorylation were inhibited by atrazine, while the PMS-dependent photophosphorylation was unaffected. *N*-Dealkylation is only a partial detoxication reaction which decreased the inhibitory activity of atrazine.

The results of this investigation imply that an interaction between three factors exists in the cells of leaf tissue to influence atrazine selectivity in higher plants. These factors are the rate of atrazine metabolism and the derivatives of atrazine formed, the concentration of the inhibitor in the chloroplasts, and the recovery of photochemical activity in the chloroplasts. A diagrammatic model of a plant cell is shown in Figure 4 to explain the proposed interaction which serves as the basis for selectivity.

Atrazine must first penetrate the chloroplast and reach the active site involved in the oxidation of water to produce an inhibitory effect. Once the inhibitor reaches this site, it must meet the steric requirements for active inhibition. Moreland (1967) has stated that the inhibitory efficiency of a compound may be related to its partitioning behavior, electron distributions, and steric factors. Good (1961) reported the reduction in inhibitory activity of substituted phenylurea herbicides when polar groups were added to the molecules. It was postulated that one of the reasons for such a reduction in activity was that changes occurred in the partitioning and penetration properties of the molecule. The more polar derivatives may fail to penetrate the lipid-rich chloroplasts in any significant amounts. Although the *s*-triazines are chemically very different from the substituted phenylureas, the solubility of atrazine and its metabolites may also be an important factor in the penetration of the lipophilic chloroplast.

Izawa and Good (1965), working with isolated spinach chloroplasts, reported that one of the absorption processes in the chloroplasts involved the partitioning of atrazine between the biological and aqueous solvent phases. Another absorption process was dependent on the concentration of the inhibitor in the medium. The absorbed atrazine in the chloroplasts consisted of a low concentration of strongly bound, inactive atrazine, and an active, reversibly bound concentration which is in equilibrium with the external solution. This inhibitory concentration was removed by washing the chloroplasts. Moreland and Hill (1962) also demonstrated the restoration of photochemical activity in turnip chloroplasts by washing the chloroplasts after treatment with simazine.

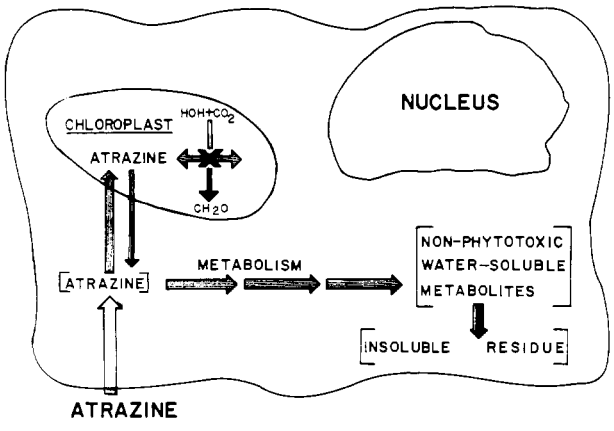

Figure 4. Diagrammatic representation outlining the proposed interaction of three important factors in a living cell

(1) atrazine metabolism, (2) inhibition of photosynthesis, (3) reduction of atrazine concentration in chloroplast and recovery of photoactivity

The information obtained from isolated systems may be translated to a living plant cell, as shown diagrammatically in Figure 4, to explain some of the observations made in intact tissues. It can be assumed that the cells in the leaf of a plant will absorb atrazine from either the vascular system or the leaf surface, if atrazine is applied on the foliage. Atrazine in the cell will then penetrate and accumulate in the chloroplasts until an equilibrium concentration is attained between the chloroplasts and the cytoplasm (equivalent to the medium in an isolated system). In this discussion the cytoplasmic concentration is not differentiated into the various cell organelles present in the cytoplasm. The degree of photosynthetic inhibition, as represented diagrammatically in the chloroplast (Figure 4), will then be a function of the active, reversibly bound atrazine concentration in the chloroplasts at equilibrium. Due to the existing equilibrium, metabolism of atrazine outside the chloroplast will have the effect of "washing" the chloroplast to reduce its atrazine concentration. The dynamic changes affecting such an equilibrium in a living cell will result in a recovery of photosynthesis from its inhibited rate over a period of time.

The results in Figure 1 reflect the degree of photosynthetic inhibition as related to atrazine concentration. The recovery of photosynthesis coupled to the metabolism of atrazine is clearly indicated in the results presented in Figure 2 and Table II. The reduction in atrazine concentration was chiefly due to the metabolism of atrazine to form the water-soluble derivative, metabolite B, and incorporation into insoluble residue.

According to the cellular model, an equilibrium should exist between atrazine concentration in the chloroplasts and the rest of the cell at any given time. Atrazine metabolism should then reduce the concentration of atrazine in the chloroplasts, but the equilibrium relationship must still be maintained. The results in intact leaves indicate that the concentration of the chloroform-soluble compounds (lipophilic compounds which include atrazine and compound I) in the chloroplasts and supernatant of sorghum and pea (Figure 3) appears to have a definite equilibrium relationship. This equilibrium seems to be maintained while atrazine concentration in the chloroplasts is progressively reduced, owing to metabolism of the inhibitor. The absence of water-soluble compounds in the chloroplasts, except for a small amount in sorghum chloroplasts at the 168-hour period, suggests that atrazine metabolism may occur outside the chloroplasts, and

the more polar compounds formed do not readily penetrate the chloroplasts. The metabolism of atrazine to more polar water-soluble compounds and insoluble residue is a highly effective process for reducing atrazine concentration in the chloroplasts, as indicated by the cellular model (Figure 4).

The question of whether the water-soluble compound(s) has the molecular structure necessary for inhibition of the Hill reaction is inconsequential, since very little of the compound is present in the chloroplasts of sorghum and pea. However, the lipophilic, *N*-dealkylated derivative, compound I, readily penetrates pea chloroplasts and is in an apparent equilibrium with the supernatant concentration (Table III). Compound I is less active than atrazine, but the mode of action seems to be the same for both compounds (Table I). Steric changes or other unknown factors may be the cause of the decreased inhibitory activity of compound I as compared to the parent atrazine, since the ability to penetrate the chloroplasts does not seem to be a factor. If the total inhibitory activity is the resultant of the activities of unchanged atrazine and compound I present in the chloroplasts, the reason that pea is intermediately susceptible to atrazine, as reported (Shimabukuro, 1967a), is readily apparent. In a highly susceptible species such as soybean, where very little *N*-dealkylation of atrazine occurs (Shimabukuro, 1967a), the inhibitor present in the chloroplasts can be expected to be predominantly unchanged atrazine.

Atrazine metabolism may cause a decrease in biological activity owing to changes in steric structure, but changes in solubility, which prevents penetration of the inhibitor into the chloroplasts, may be of even greater significance. The basis for resistance in corn may be the same as for sorghum. Hydroxyatrazine is rapidly formed as the predominant water-soluble metabolite in corn, with small amounts of hydroxycompounds I and II present (Shimabukuro, 1968). The partitioning of these derivatives between the chloroplasts and supernatant of corn has not been shown, but the change in solubility due to metabolism suggests a similar process occurring in corn. Solubility of atrazine in chloroform is 0.24 mole per liter, while the hydroxylated derivatives are insoluble in the same solvent.

The atrazine-sensitive site seems to be the same for resistant as for susceptible plants. The ability to metabolize atrazine and, thereby, reduce the active concentration of the inhibitor at the primary site of action seems to be the basis for selectivity in higher plants.

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