SYMPOSIUM ON DECOMPOSITION AND METABOLISM OF HERBICIDES

Introduction

The continuing and rapid development of new and effective herbicides required to meet the increasing demands of agriculture to feed an ever-expanding populace has led to a concomitant expansion in studies on the decomposition and metabolism of these herbicides. Such studies have made a significant impact upon our understanding of the manner in which nature and living systems cope with new inputs into their environment. These multidisciplinary investigations have given us new insights into the comparative biochemistry of plants, animals, and microbial systems, and provided us with more useful scientific rationale for the design of better herbicides. The research findings exemplified in this symposium should also permit us to develop a more precise and informed understanding of the influence of herbicides upon our environment and aid in the objective analysis of potential hazards.

The specific objective of this symposium was to cover recent developments regarding the decomposition and metabolism of the newer herbicides in plants and in soil, as well as to bring scientists up to date on the progress of metabolism and decomposition of herbicides in general. Consideration was given both to biological factors and to chemical and physical factors. Soil decomposition of herbicides was considered from the microbiological aspect as well as from purely physical aspects involving adsorption of herbicides in the soil environment. Photodecomposition of herbicides was also discussed since this phenomenon may play an important role in the nature of the nonbiological modification of herbicides, a factor that may be of considerable importance in the interpretation of "apparent biological" degradation.

The Pesticide Subdivision of the Division of Agricultural and Food Chemistry can be of real service to scientists by continuing to sponsor such interdisciplinary symposia in the area of pesticide metabolism, degradation, and mode of action.

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Significance of Atrazine Dealkylation in Root and Shoot of Pea Plants

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The major metabolite of atrazine in both roots and shoots of young pea plants was 2-chloro-4-amino-6-isopropylamino-*s*-triazine (compound I). Both roots and shoots were able to metabolize atrazine independently to compound I. The phytotoxicity of compound I was less than that of atrazine. The conversion of the highly toxic atrazine to the less toxic metabolite, compound I, and the accumulation of these compounds in the plant may be a mechanism resulting in intermediate susceptibility of pea plants to atrazine.

The major metabolite of 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) in mature pea plants was recently isolated and identified as the dealkylated product, 2-chloro-4-amino-6-isopropylamino-*s*-triazine (compound I) (*17*). A dealkylation product of 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) was also identified in the culture media of the soil fungus, *Aspergillus fumigatus* (Fres.), as 2-chloro-4-amino-6-ethylamino-*s*-triazine (*11*). Previous reports on metabolism of atrazine and simazine in higher plants have identified the major degradation products as 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine (hydroxyatrazine) and 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxysimazine) (*1*, *7*, *8*, *14*, *16*).

The tolerance of corn, *Zea mays* (L.), to atrazine and simazine is believed to be due largely to its ability to degrade simazine (*1*, *6*, *8*, *16*) and atrazine (*14*) to hydroxysimazine and hydroxyatrazine, respectively. Plants are believed to respond similarly toward atrazine and simazine. Other species besides corn have been reported to metabolize atrazine (*15*) and simazine (*7*) to their hydroxy derivatives. The ability of plants to convert the 2-chlorotriazines to their 2-hydroxy derivatives was reported to be correlated with the presence of a cyclic hydroxamate, 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine (benzoxazinone) in plants (*7*).

Attempts have been made to explain intermediate susceptibility or tolerance among different species to the triazines. The amount of simazine absorbed does not determine susceptibility of a given species (*3*). A correlation between the amount of unchanged atrazine found in plants and susceptibility has been reported for several plant species (*15*). However, sorghum, a resistant species, did not metabolize simazine to hydroxysimazine (*7*) while the moderately susceptible pea plant rapidly metabolized atrazine to compound I (*17*).

In this investigation, the metabolism of atrazine in root and shoot tissues of young pea seedlings was determined. The significance of metabolism in different

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organs of a plant and the role of dealkylation in explaining intermediate susceptibility or tolerance to atrazine are discussed.

Materials and Methods

Plant Material. Pea seeds, *Pisum sativum* (L.) variety Little Marvel, were surface-sterilized in 1% sodium hypochlorite and germinated between moist paper towels in an incubator at 25° C. for 4 days. The young seedlings were transferred directly to 500 ml. of continuously aerated one-half strength Hoagland's nutrient solution in polystyrene containers. When not otherwise specified, the pea seedlings were grown and treated in a controlled environment room with a 12-hour photoperiod, light intensity of 1400 foot-candles, 22° ± 2° C. day temperature, 16° ± 2° C. night temperature, and a relative humidity of 40 ± 5%.

In phytotoxicity studies, 10 germinated seedlings were transferred directly into each container with concentrations of 10^{-7}, 10^{-6}, and $10^{-5}M$ atrazine or compound I in the nutrient solution. Nutrient solution or distilled water was added to the containers periodically to maintain the solution level. There were five containers per concentration for each compound. After 19 days, the fresh weights of roots and shoots of plants in each container were determined separately.

The relative susceptibility of pea plants to atrazine as compared with that of the highly susceptible oat, *Avena sativa* (L.) variety Rodney, was determined by comparing length of time required to kill plants of both species in $10^{-5}M$ atrazine solution. Pea plants were exposed to atrazine as mentioned above. Oat seeds were germinated for 4 days and seedlings were grown in one-half strength Hoagland's solution for another 4 days before exposure to $10^{-5}M$ atrazine solution.

Degradation of Atrazine-C^{14}. The ability of roots and shoots to metabolize atrazine independently and the effect of pretreatment with atrazine on subsequent metabolism of the herbicide were studied in pea plants. Twenty-four control plants and plants exposed to 5 p.p.m. atrazine for 7 days (pretreated plants) were subsequently exposed for 48 hours to 0.36 μc. of uniformly ring-labeled atrazine-C^{14} (specific activity 7.8 μc. per mg.) in 300 ml. of aerated Hoagland's solution. Control plants were not exposed to unlabeled atrazine prior to exposure to atrazine-C^{14}. Pea seedlings referred to as pretreated plants were transferred directly into atrazine solution after the 4-day germination period. Unlabeled atrazine was added to the treatment solution with radioactive atrazine to make the final concentration of atrazine equal to 5 p.p.m. Atrazine-C^{14} used in the experiments was purified by thin-layer chromatography. Assay of C^{14} activity in purified atrazine-C^{14} showed less than 0.6% of the radioactivity present as impurities. Plants were allowed to absorb and translocate atrazine-C^{14} in a laboratory hood under the conditions previously reported (*17*).

To determine the metabolism of atrazine in roots independently of shoots, the epicotyls of germinated seedlings were excised immediately upon transfer into nutrient solution. Only the root and cotyledons were left intact. These roots are referred to in the text as decapitated roots. Growth studies indicated that removal of epicotyls did not affect the growth of decapitated roots over an 11-day period. The dry weight of control decapitated roots increased from 5.2 mg. per root at time of excision to 43.8 mg. per root after 11 days. Roots from intact plants weighed 46.1 mg. per root after the same period. Both control and pretreated decapitated roots were exposed to atrazine-C^{14} as with intact plants after 7 days of pretreatment.

Two different methods of exposure to atrazine-C^{14} were used to investigate the metabolism of atrazine-C^{14} in shoots independent of roots. The shoots of eight control and pretreated plants were excised under water just above the cotyledons, and the cut ends were immersed in 40 ml. of distilled water containing 0.27 μc. of atrazine-C^{14}. These shoots, which remained fresh and turgid throughout the 48-hour exposure period, are referred to as excised shoots in the text. In a second method the distal one third of both cotyledons of intact control and pretreated plants were excised and only the cut surfaces of the cotyledons were exposed to 0.14 μc. of atrazine-C^{14} in 1 ml. of aqueous solution. In the text these plants are referred to as cotyledon-fed plants. Distilled water was added to replace water loss and to keep the cut surfaces of cotyledons immersed in the aqueous solution. Exposure time was 48 hours and treatment conditions were the same as above.

To determine whether translocation out of cut cotyledons occurred through the xylem, the stem immediately above the cotyledons was steamed in two control plants, and the hypocotyl immediately below the cotyledons was steamed in another pair of plants. Twenty-four hours later the cotyledons of steamed and unsteamed plants were exposed to atrazine-C^{14} as above. In a similar set of plants, uncut whole cotyledons were exposed to atrazine-C^{14}. After 48 hours, the roots of cotyledon-fed plants were thoroughly rinsed, and plants were cut into several parts, dried at 70° C. for 24 hours between blotter papers, and placed against x-ray film for autoradiography.

Extraction and Assay for C^{14} Activity. After 48 hours of exposure to atrazine-C^{14}, the root and shoot tissues were extracted separately with 95% methanol and the extracts purified as previously reported (*17*). Methanol was evaporated under vacuum, the plant residue was removed by centrifugation, and the resulting aqueous solution was concentrated for further assay. The aqueous extract was washed with chloroform to remove atrazine, compound I, and other chloroform-soluble metabolites. The C^{14} activity in chloroform-soluble and water-soluble compounds was determined by liquid scintillation counting (*17*).

Unchanged atrazine and compound I were measured quantitatively by spotting a known amount of radioactivity from chloroform-soluble radioactive compounds on a 5 × 20 cm., 250-micron thick silica gel HF thin-layer plate. The thin-layer chromatogram was developed in benzene–acetic acid (50 to 4). Atrazine-C^{14} (R_f 0.37) and compound I (R_f 0.26) were detected with a strip scanner. The silica gel from zones including the origin, unchanged atrazine, and compound I was care-

fully removed and added to 20 ml. of gel scintillation liquid (4% Cab-O-Sil in solution of 5.0 grams of PPO and 0.3 gram of dimethyl POPOP per liter of toluene) and assayed by gel scintillation counting. Between 85 and 90% of the C^{14} activity originally applied to the thin-layer plate was recovered. All samples were corrected for quenching and the results expressed as disintegrations per minute (d.p.m.).

The C^{14} activity remaining in the extracted plant residue was measured by dry combustion in Schöniger flasks and assayed by liquid scintillation counting (*12*). The extracted plant residue was air-dried and ground in a Wiley mill to pass a 40-mesh screen. From 80 to 110 mg. of the homogeneous ground material was burned in oxygen in a 1-liter Schöniger flask. After cooling the flask in an ice bath, 10 ml. of Hyamine hydroxide 10-X (1*M*) was added to the cooled flask, which was swirled vigorously and allowed to stand at room temperature for 20 minutes. One milliliter of Hyamine hydroxide 10-X was removed and added to 15 ml. of scintillation counting liquid (toluene scintillation liquid without Cab-O-Sil) for assay. This procedure gave 94 to 98% recovery of a known amount of atrazine-C^{14} burned with ground nonradioactive plant residue.

Results

A cotyledon-feeding method was developed to study the metabolism of atrazine in the shoot of an intact plant independent of metabolism in the root. The objective was to introduce atrazine-C^{14} into the shoot in its unchanged form while bypassing the normal path from root to shoot.

Radioactivity from atrazine-C^{14}, absorbed through the cut surfaces of cotyledons, was readily detected in the shoot of pea plants (Figure 1, left). The autoradiogram of the plant indicates a general distribution pattern characteristic of xylem transport. To eliminate phloem transport, the stem section above the cotyledons was steamed prior to exposure of cut surfaces of cotyledons to atrazine-C^{14}. Although not shown in Figure 1, a similar general distribution pattern was observed in unsteamed plants and plants in which a section of the hypocotyl immediately below the cotyledons had been steamed. No radioactivity was present in the roots of any of the above plants.

When whole, uncut cotyledons of similarly steamed and unsteamed plants were exposed to atrazine-C^{14}, no radioactivity was detected in the shoot or root in any of the plants. Radioactivity was present only in the whole cotyledons which absorbed atrazine-C^{14} (Figure 1, right).

The results indicated that export of atrazine-C^{14} or its metabolites from cotyledons occurred only when atrazine-C^{14} was absorbed through cut surfaces of cotyledons. Translocation only in the acropetal direction in steamed plants indicated that transport out of cut cotyledons occurred via the xylem.

When atrazine-C^{14} is absorbed directly through the surface of whole, uncut cotyledons, it is expected that penetration into parenchyma cells and subsequent "loading" onto the phloem are necessary for translocation out

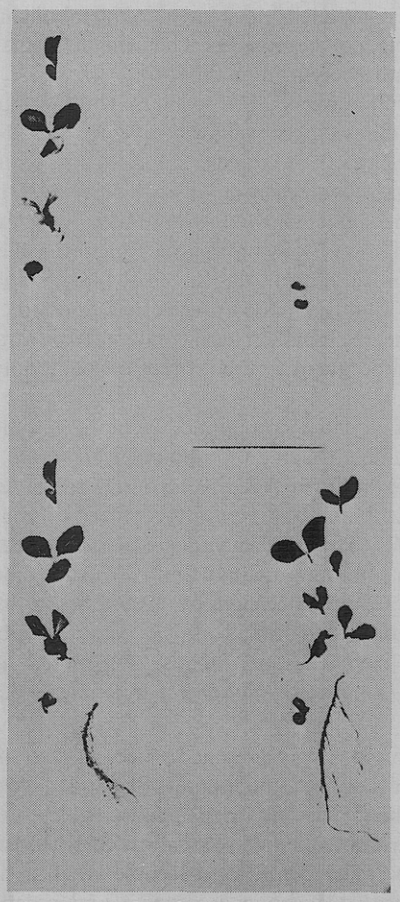

Figure 1. Cotyledon-fed pea plants

Top row. Autoradiograms of plants below. *Left.* Atrazine-C^{14} absorbed through cut surfaces of cotyledons and stem section above cotyledons steamed. *Right.* Whole uncut cotyledons exposed to atrazine-C^{14} and stem not steamed

of cotyledons. The absence of radioactivity in shoot and root when whole, uncut cotyledons were exposed to atrazine-C^{14} indicated the absence of phloem transport. This agreed with reports for simazine, as well as atrazine, which indicated that the chlorotriazines are predominantly translocated in the xylem and not the phloem (*2*).

If translocation of atrazine-C^{14} is to occur out of cotyledons, the compound must be introduced directly into the xylem and bypass parenchyma cells of the cotyledons. This is made possible by cutting the cotyledons and exposing the ends of xylem vessels present in pea cotyledons (*9*). The results obtained when cut surfaces of cotyledons were exposed to atrazine-C^{14} suggest that atrazine-C^{14} was absorbed through the exposed ends of xylem vessels and translocated into the shoots via the xylem.

If radioactive compounds are translocated out of cotyledons only when absorbed through exposed xylem vessels, it is reasonable to assume that atrazine-C^{14} is being translocated to the shoot in the unchanged form. Thus, the cotyledon-feeding method can be used to study the metabolism of atrazine in the shoot of an in-

tact plant completely independent of metabolism in root. This would not be possible when intact plants are exposed to atrazine through the root.

Decapitated roots grew normally with an increase in fresh and dry weights over an 11-day period. The cotyledons apparently contained sufficient reserve material to support growth of roots over this period. In intact plants, the shoots showed symptoms of atrazine injury at the end of the 7-day pretreatment period. The leaves were chlorotic but not desiccated. The metabolism of atrazine-C^{14} after a 7-day pretreatment period could reflect the degree to which herbicidal injury by atrazine influences subsequent metabolism of the compound in the pea plants.

The dealkylated compound, 2-chloro-4-amino-6-isopropylamino-*s*-triazine (compound I, R_f 0.26) was the major metabolite of atrazine in young pea plants. This agreed with this author's results reported for mature pea plants (*17*). However, in younger plants a trace amount of 2-chloro-4-amino-6-ethylamino-*s*-triazine (compound II, R_f 0.22) was detected by autoradiography. This compound was identified as the major metabolite of simazine from the soil fungus, *Aspergillus fumigatus* (Fres.) (*11*), but it was not detected in mature pea plants (*17*).

Compound I was present in decapitated roots and roots and shoots of intact plants (Table I). More than 80% of the C^{14} activity in the plant samples, except for roots from control intact plants, was chloroform-soluble (Table I). The chloroform-soluble fraction included predominantly unchanged atrazine and compound I. The shoots from intact control plants showed nearly equal amounts of atrazine and compound I present in the chloroform-soluble fraction (45.8% metabolite and 49.6% atrazine). All root samples, shoots from intact pretreated plants, and excised shoots showed lower amounts of compound I than atrazine (Table I).

Pretreatment slightly reduced the ability of plant organs to dealkylate atrazine. In decapitated roots, herbicidal injury resulted in a decrease of 6.4% in the amount of compound I formed, or an accumulation of 7.3% more atrazine as compared to the controls. The results on decapitated roots suggest that atrazine has a direct physiological effect on nonphotosynthetic root tissues rather than a secondary effect resulting from initial injury to shoots. The reduction in growth caused by atrazine on nonphotosynthetic tobacco callus tissue also supports the suggestion of a direct effect (*10*).

In intact plants, the effect of pretreatment was greater than in decapitated roots. The injured roots showed 11.1% reduction in the amount of compound I formed, or an accumulation of 12.7% more atrazine than the controls. The injured shoots from the same plants showed 8.9% reduction in the amount of compound I formed, or an accumulation of 10.7% more atrazine than the controls (Table I). The results suggest that herbicidal injury to plants caused by long exposure to high concentrations of atrazine may reduce the ability of pea plants to metabolize the compound.

The high percentage of chloroform-soluble radioactive compounds as compared with water-soluble compounds indicates that an accumulation of compound I and atrazine occurs in the plant organs. Very little metabolism of atrazine-C^{14} beyond dealkylation may occur within 48 hours, and this is substantiated by the low percentage of radioactivity recovered as water-soluble compounds and methanol-insoluble plant residue (Table I). The methanol-insoluble residue was slightly higher in roots than in shoots.

Table I. Distribution of Radioactivity in Decapitated Roots, Roots and Shoots of Intact Pea Plants, Excised Shoots, and Shoots of Cotyledon-Fed Pea Plants Exposed to Atrazine-C^{14} for 48 Hours

Sample	Chloroform-Soluble,[a] %	Radioactivity in Chloroform-Soluble Fraction, %		Methanol-Insoluble Residue, %
		Compound I	Atrazine	
Decapd.[b] roots (PT)[c]	80.5	23.0	71.5	8.5
Decapd. roots (C)[d]	80.5	29.4	64.2	8.7
Intact roots[e] (PT)	81.8	23.7	71.5	7.9
Intact roots (C)	74.7	34.8	58.8	11.9
Intact shoots (PT)	86.0	36.9	60.3	2.0
Intact shoots (C)	84.3	45.8	49.6	2.6
Excised shoots[f] (PT)	87.5	30.8	67.6	1.5
Excised shoots (C)	89.3	30.0	69.2	1.8
Cot.-fed shoots[g] (PT)	84.8	52.6	45.1	2.6
Cot.-fed shoots (C)	89.9	59.0	38.8	1.9

[a] Includes metabolite (compound I) and atrazine. Water-soluble fraction does not contain hydroxyatrazine. [b] Epicotyls excised (decapitated) immediately upon transfer of germinating seedlings into nutrient solution. [c] Pretreated (PT) with nonradioactive atrazine for 7 days. [d] Control (C) plants not exposed to nonradioactive atrazine. [e] Intact roots and shoots are from same plants but assayed separately. [f] Shoots from intact plants excised just prior to exposure to atrazine-C^{14}. [g] Shoots from pretreated (PT) and control (C) intact plants where atrazine-C^{14} was introduced through cut surfaces of cotyledons (cotyledon-fed). Roots were not assayed.

Compound I was also the major metabolite in excised shoots and shoots of cotyledon-fed pea plants (Table I). Pretreatment also reduced slightly the metabolism of atrazine-C^{14} in shoots of cotyledon-fed plants. These shoots showed a reduction of 6.4% in the amount of compound I formed or an accumulation of 6.3% more atrazine than the controls. In excised shoots the metabolism of atrazine-C^{14} was considerably less than shoots of cotyledon-fed plants. The amount of compound I formed was 21.8% less in pretreated excised shoots than in pretreated cotyledon-fed shoots. The equivalent control samples showed a reduction of 29.0% in the amount of compound I formed.

Control and pretreated excised shoots did not differ in metabolism of atrazine-C^{14} (Table I). Apparently, physiological injury caused by complete separation of shoots from roots greatly reduced the ability of excised shoots to metabolize atrazine, as compared with shoots of cotyledon-fed plants Because of injury caused by

excision, the reduction in metabolism of atrazine-C^{14} owing to pretreatment was not apparent in excised shoots. This was not the case in decapitated roots, roots from intact plants, shoots from intact plants, and shoots from cotyledon-fed plants (Table I).

No reasonable explanation could be made for the higher percentage of compound I present in shoots of cotyledon-fed plants as compared with shoots of intact plants (Table I). The results in shoots of cotyledon-fed plants agree with the high percentage of compound I found in shoots of mature pea plants (*17*). Apparently, atrazine and compound I accumulate in leaf tissues and are not transported out into other organs. High concentrations of compound I and atrazine were found in shoots of cotyledon-fed plants, but no radioactivity was detected in the roots (Figure 1, left).

The relative phytotoxicities of atrazine and compound I on pea plants were determined by measuring the per cent of inhibition of fresh weight at three concentrations (Table II). After 19 days, plants in $10^{-7}M$ atrazine or compound I showed very little injury. Plants in $10^{-6}M$ atrazine or compound I were lightly chlorotic and slightly stunted in growth. Severe injury to plants appeared only in $10^{-5}M$ atrazine or compound I (Figure 2). The shoot apex of plants in $10^{-5}M$ atrazine was dried and dead. Severe chlorosis and stunting occurred in $10^{-5}M$ compound I, but the plants were still turgid and fresh.

Pea plants can tolerate low concentrations of atrazine and compound I. No difference in toxicity appeared between atrazine and compound I at 10^{-7} and $10^{-6}M$ concentrations (Table II, Figure 2). However, atrazine was more toxic than compound I at $10^{-5}M$ concentration. At this concentration, atrazine reduced fresh weights of shoots and roots by 78.6 and 70.0%, respectively. Compound I gave 58.0 and 67.2% reduction in fresh weights of shoots and roots (Table II).

The higher tolerance of pea plants than a highly susceptible species to atrazine was demonstrated by comparing the time required to kill pea and oat plants in $10^{-5}M$ atrazine solution. It required 21 days to kill pea plants, whereas the highly susceptible oat plants were killed within 7 days. The results indicate that young pea plants are tolerant or intermediately susceptible to atrazine. Compound I, the major metabolite of atrazine, was phytotoxic to pea plants but was less toxic than the parent compound.

Table II. Inhibition of Fresh Weight in Pea Plants from 19-Day Exposure to Atrazine and Compound I

Compound and Organ	Inhibition of Fresh Weight at Varying Molar Concentrations,[a] %		
	10^{-7}	10^{-6}	10^{-5}
Atrazine			
Shoot	14.0	32.5	78.6
Root	4.3	35.5	70.0
Compound I			
Shoot	13.2	30.0	58.0
Root	3.2	32.2	67.2

[a] Average of five replicates with 10 plants per replicate.

Discussion

In young pea plants the roots and shoots have the ability to metabolize atrazine independently to compound I. The shoots showed a higher capacity to metabolize atrazine than did roots. Under normal conditions, the roots are involved in uptake of atrazine from the root environment and release of the compound into the xylem for translocation upward into the shoot. In the uptake process the root cells metabolize part of the atrazine absorbed to compound I. Since shoots of plants exposed to $10^{-5}M$ compound I showed signs of injury, it is likely that compound I was translocated from the root to shoot. Therefore, in plants exposed to atrazine both unchanged atrazine and compound I formed in the root may be translocated to the shoot.

Cotyledon-feeding experiments showed that the shoot does not transport atrazine or compound I to the root because neither compound is phloem-translocatable. Therefore, in an intact plant the concentration of compound I in the root is entirely due to metabolism of atrazine in the root tissue. In the shoot part of the compound I concentration is made up of translocated compound I formed in the root.

Pretreatment of plants with a phytotoxic atrazine concentration reduced slightly the ability of plant organs to dealkylate atrazine. The reduction was slightly greater in roots than in shoots. This was observed by comparing the percentage of compound I and unchanged atrazine present in roots and shoots of control and pretreated intact plants, decapitated roots, and cotyledon-fed shoots (Table I). Whether root tissues are more sensitive than shoot tissues to herbicidal injury requires further study. The reduction in dealkylation in pretreated plants may also be due to concentration effects resulting from dilution of atrazine-C^{14} with unlabeled atrazine absorbed during the 7-day pretreatment period. Physiological injury and concentration effects may both contribute to a reduction in dealkylation.

Atrazine and compound I accumulate in pea plants. The small amount of atrazine metabolized to water-soluble compounds and methanol-insoluble residue and the absence of hyroxyatrazine in pea plants (*17*) suggest that very little metabolism beyond dealkylation occurs in pea plants within 48 hours. A degradation scheme for atrazine and simazine, with the 2-hydroxy derivatives

Figure 2. Photograph of pea plants taken 19 days after initial exposure to atrazine and compound I

Left to right. Control, $10^{-6}M$ atrazine, $10^{-6}M$ compound I, $10^{-5}M$ atrazine, and $10^{-5}M$ compound I

of the 2-chlorotriazines as the initial intermediate metabolite, has been proposed for higher plants (*6*, *13*). This scheme is based primarily on results obtained in corn where hydroxyatrazine and hydroxysimazine are the predominant metabolites of atrazine and simazine. In pea plants the degradation pathway for atrazine does not seem to be according to the scheme proposed for corn.

Pea plants were not as susceptible as oats to atrazine. Although stunting occurred at lower concentrations of atrazine, pea plants were tolerant of atrazine concentrations at less than $10^{-6}M$. Compound I was also definitely less phytotoxic than atrazine to the pea. The significance of the dealkylation reaction becomes apparent when it is realized that a plant which is discovered to be intermediate in susceptibility to a given compound is capable of converting the highly toxic compound, atrazine, rapidly to a less toxic derivative, compound I.

The author proposes that in pea plants there is a mechanism which results in intermediate tolerance of the species to atrazine. The conversion of inactive compounds to biologically active compounds in plants is well documented (*4*, *5*, *18*, *19*). The oxidation of phenoxybutyric acids by β-oxidation to form herbicidally active phenoxyacetic acid has been demonstrated (*5*, *19*). An example wherein a slight change in chemical structure results in the conversion of a herbicidally active compound to an inactive form has been demonstrated with two closely related herbicides, atrazine and simazine. The metabolism of 2-chlorotriazines to the 2-hydroxytriazines is a well known reaction resulting in complete detoxication of a herbicide in higher plants (*1*, *8*, *14*, *16*). Dealkylation of atrazine in pea plants is neither an activation reaction nor a complete detoxication reaction as demonstrated in the examples mentioned above, but is intermediate in activity. Dealkylation of atrazine to form compound I is an example in which a highly active compound is converted to a form with lower activity. The total herbicidal effect on pea plants exposed to atrazine would be the resultant of the phytotoxicities of unchanged atrazine and compound I. The resultant phytotoxicity can be expected to be lower than the phytotoxicity of unchanged atrazine itself.

The conversion within a plant of a highly toxic herbicide to a less toxic metabolite may be a mechanism resulting in intermediate tolerance or susceptibility of different species to a given herbicide. In pea plants the accumulation or failure to metabolize compound I rapidly to a completely nonphytotoxic form may keep the plants from being completely resistant to atrazine. Sorghum does not convert atrazine to nonphytotoxic hydroxyatrazine (*7*), but it is known to be a resistant species. Dealkylation of atrazine with subsequent rapid metabolism of the dealkylated product to a nontoxic form could be another mechanism of resistance to atrazine. The possibility that such a mechanism exists in sorghum is now under study.

Acknowledgment

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