The Effect of Soil Residues of Atrazine on Sugarbeets (*Beta vulgaris* L.)*

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INTRODUCTION

Sugarbeets are often damaged by soil residues of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] left after treatment of corn. Frank (3) reported that carryover of atrazine in Southern Ontario, Canada was greater when atrazine was applied postemergence rather than preemergence to a preceding crop of corn. Broadcast applications and 2 lb ai/A (as opposed to 1.5 lb ai/A) were more detrimental than bands. When sugarbeets followed corn, treated two years previously with atrazine, injury was correlated only with rate of application. Soybeans (*Glycine max* L.), navy beans (*Phaseolus vulgaris* L.) and oats (*Avena sativa* L.) were all less sensitive than sugar-beets.

One objective of this study was to determine the levels of residual atrazine that cause injury to a succeeding crop of sugarbeets in greenhouse and field experiments. A second objective was to correlate the results of chemical and biological analyses for atrazine. Eberle and Gerber (2) found that chemical and bioassay analysis for degradation rates of ametryn [2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine], a phenyl urea, and 2,4-D [(2,4-dichlorophenoxy)acetic acid] showed a correlation coefficient of 0.914. No correlation was established between the two methods of analysis for fluorodifen

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(*p*-nitrophenyl α,α,α-trifluoro-2-nitro-*p*-tolyl ether). Comparative studies on atrazine residues and specific bioassays for atrazine with sugarbeets were not found but other bioassay procedures for atrazine were described by Behrens (1).

A biological assay for atrazine takes a minimum of two weeks whereas a chemical assay may be performed in one day. Because the question of atrazine residue often arises in the spring just prior to planting, it would be beneficial to have a more rapid method of analysis than that provided by biological assay. In addition, the most common bioassay for atrazine in soil utilizes tame oats as the test plant (1), and these data have not been correlated with the response of sugarbeets. Bioassays rest on the assumptions that (1) there is a linear plant response to dose and (2) the response is reproducible under the same test conditions (1, 2). These assays are most effective for determining phytotoxic but not total residues. The opposite is true for chemical methods which determine total residues but give no indication of their phytotoxicity (2).

MATERIALS AND METHODS

BIOASSAY STUDIES

Because the response of sugarbeets to atrazine had not been quantified, we began with the hypothesis that it would approximate the limits of 0.04 to 2.5 lb ai/A used in the oat bioassay. A standard concentration series was prepared by treating each of three soil types, Ascalon, Weld, and Heldt (Table 1), with a solution of atrazine in benzene. Within this concentration range all sugarbeets died within three weeks. Concentrations of atrazine in subsequent experiments ranged from 0.0031 to 0.6 ppmw. All soils were treated with 0.6 ppmw of atrazine and lower concentrations were obtained by soil dilution. Sufficient soil for the entire experiment was mixed as one batch for a minimum of one hour in a twin shell "V" blender.

Table 1

Soils used for atrazine bioassay study.

Soil textural class	sand	silt	clay	O.M.	CEC
	%				(meq/100g)
Sandy loam	77	11	12	0.6	7.8
Loam	31	45	24	2.0	15.8
Clay loam	24	36	40	2.2	30.5

Treated soil was stored dry in covered glass jars until needed. Seven ounce styrofoam drinking cups, with holes in the bottom for drainage, were used as pots. After soil was added to the cups they were placed in a large tray in a randomized block design with five replicates and sub-irrigated until completely wet. Great Western Mono-Hy D-2 sugarbeet seeds were prepared for planting by wrapping them in paper towels and soaking them under cold, running water for about one hour. The seeds were then transferred to a dry paper towel and allowed to air-dry for about 15 minutes. The partially dried seeds were treated with thiram (tetramethylthioramidisulfide) by shaking in a small plastic bag. Ten or more seeds were planted per cup, and the surface of each cup was then covered with styrofoam beads to reduce moisture loss. After emergence plants were thinned to five per cup. Earlier experiments had shown that length of the first true leaf (blade and petiole) was a reliable growth measurement to predict atrazine presence. Sugarbeets were grown 18 days in Heldt and Ascalon soil, but it took 29 days to reach the same growth stage in Weld soil. At these times the length of the first true leaves for five plants per pot was measured.

FIELD STUDIES

To relate the effect of atrazine on sugarbeet growth in the greenhouse to that observed in the field, a two-year field experiment was performed. Corn was planted on May 15, 1975 in a randomized block with four replications.

Atrazine was applied preemergence four days later at rates of 2.0, 1.0, 0.5, 0.25, 0.125 and 0 lb ai/A. All plots were hoed several times during the growing season to control annual and perennial weeds. In October, the center two rows of corn were hand harvested from each plot. Fresh and dry weights were recorded and yield of corn silage was calculated in tons per acre.

In April, 1976, after plowing to about 10 inches, Mono-Hy D-2 sugarbeet seeds were planted in the same plots. After emergence sugarbeets were thinned to one plant per foot of row. Sprinkler irrigation was used and hand weeding employed as necessary in all plots to prevent excessive weed competition. Visual injury ratings were made during the season. In October, the center two rows of sugarbeets in each plot were harvested. Roots were topped, washed, weighed, and two random samples of 15 roots were analyzed for purity and sucrose.

CHEMICAL STUDIES

To determine total residual concentrations of atrazine present in soil at the time sugarbeets were grown, soil samples were chemically analyzed. Soil samples from each plot were taken in May, 1975 soon after initial atrazine treatment. Samples were taken again at corn harvest (October, 1975), after sugarbeets were planted (April, 1976) and following sugarbeet harvest (October, 1976). All samples were frozen for later extraction and analysis.

Atrazine was extracted from soil by refluxing 50 g for one hr in 90% acetonitrile/water (v/v). The extract was added to a separatory funnel and extracted with two 25 ml portions of methylene chloride, which were combined, dried, and transferred to an alumina column for clean up. After elution with benzene-ether (60:40) samples were brought to an appropriate volume in benzene for analysis.

Atrazine was detected using an electron capture gas

chromatograph and a Dohrmann-Envirotech halogen specific microcoulometer. Analysis with electron capture showed that atrazine could be detected with a linear range of 1-10 nanograms (Ng). However, even after sample clean up, many impurity peaks resulted, so only the microcoulometer was used. Operating conditions were:

Injection port temperature	215°C
Column	200°C
Transfer section	280°C
Combustion oven	800°C
Outlet section	720°C
Argon flow rate	40 ml/min
Oxygen flow rate	100 ml/min

A 30 cm long 2 mm ID glass column was packed with 5% SE-30 on 60/80 mesh gas-chrom Q. On low gain with a range of 300 ohms the linear range of detection was 10 to 100 Ng. Injection volumes from 2 to 10 μl were used.

RESULTS AND DISCUSSION

BIOASSAY STUDIES

An atrazine concentration of 0.2 ppmw or higher killed sugarbeets, while 0.1 ppmw atrazine seriously affected sugarbeet growth in all three soil types (Table 2). Data are expressed as percent of the untreated control rather than length of the first true leaf and represent the average of several experiments conducted in each soil type. Curves showing percentage of control growth vs. applied atrazine concentration were drawn for each soil. From the regression equations obtained for these curves, the percentage decrease in sugarbeet growth for any applied atrazine concentration was calculated (Table 3). Although these data are important, they have little practical value except as a general guideline, as they are based on the quantity of atrazine applied rather than on the amount which is available to affect sugarbeet growth. The relationship between the two has not been determined. Nevertheless, it is significant that growth was suppressed 50% in all three soils at

Table 2

Growth of sugarbeets in the greenhouse in three soils treated with atrazine.

Atrazine concentration in soil (ppmw)	Growth as % of untreated control[a]		
	sandy loam	loam	clay loam
0	100a	100a	100a
0.0031	89b	56b	119a
0.0063	83b	58b	92a
0.0125	82b	55b	82b
0.025	84b	20c	30c
0.05	68c	16c	11d
0.1	17d	5d	2d
0.2	0	0	0
0.4	0	0	0
0.6	0	0	0

[a] Values in one column followed by the same letter are not significantly different at the 1% level of probability according to Duncan's multiple range test.

Table 3

Calculated concentration of atrazine required to decrease sugarbeet growth in greenhouse bioassay studies.

Soil	Growth suppression	Atrazine
	(%)	(ppmw)
Sandy loam	10	.0050
	25	.0150
	50	.0590
Loam	10	.0148
	25	.0184
	50	.0272
Clay loam	10	.0063
	25	.0087
	50	.0163

atrazine concentrations of 0.059 ppmw or less and that there was a difference between soils. Fifty percent growth suppression occurred at 0.059, 0.0272 and 0.0163 ppmw in sandy loam, loam, and clay loam soil, respectively. This relationship is not surprising given the well documented adsorptive characteristics of soils with higher amounts of clay and organic matter which decrease herbicide effects. Reflective of greater variability and the lack of precision in measuring small amounts the same consistency between soils was not shown for 10 and 25% growth suppression.

FIELD STUDIES

Corn yields from each atrazine treatment and the untreated plots were not significantly different (Table 4). As determined by visual injury ratings, there was slight damage to sugarbeets in plots treated with the lowest three rates of atrazine and extensive damage in plots treated with 1.0 and 2.0 lb ai/A. Atrazine applied at 1 or 2 lb ai/A decreased sugarbeet yield but did not affect percent sucrose or purity. Root yields from plots treated with 0.25 and 0.5 lb ai/A were not different from the control while a slight and unexplained increase over the control weight was found for 0.125 lb ai/A atrazine. The two highest rates decreased yield more than 50%.

CHEMICAL STUDIES

Chemical analysis is necessary to relate the amount of atrazine applied in the bioassay and in field plots. The data in Tables 2 and 3 show that extremely small quantities of atrazine greatly decrease growth of sugarbeets in bioassay studies. The two highest rates of applied atrazine reduced sugarbeet yield in the field (Table 4). The limit of chemical detection capability using the microcoulometer was 0.1 ppmw of atrazine present in soil. Lower levels of atrazine can be detected by extracting larger amounts of soil and by employing extraordinary analytical care. These measures were beyond the scope of the present study. Without extensive clean-up and analytical techniques beyond our capability, we could not

Table 4

The affect of atrazine on corn yield and sugarbeet yield.

Atrazine applied 1975	Corn silage yield 1975	Sugarbeets - 1976			
		Visual injury	Sucrose	Purity	Yield[b]
(lb ai/A)	(T/A)	(%)	(%)	(%)	(T/A)
0	3.4a	0	18 a	95.8a	24.1b
0.125	3.2a	15	17.7a	95.4a	26.0a
0.25	3.3a	8	18.1a	95.8a	24.7ab
0.5	3.6a	15	17.8a	95.4a	23.8b
1.0	3.3a	65	16.4a	93.9a	11.3c
2.0	3.7a	98	17.3a	95.1a	10.3c

[a] 0 = no injury, 100 = complete kill of sugarbeets. Average of visual ratings over several observation dates.

[b] Means followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test.

detect atrazine levels at or below 0.05 ppmw or approximately 10 Ng in 50 g of soil. Eberle and Gerber (2) chemically detected 0.04 ppm of ametryn, and biologically detected 0.2 ppm with tame oats and 0.02 ppm with Chlorella pyrenoidosa. Thus, their chemical sensitivity was about equal to ours but their bioassay species were not as sensitive. In sugarbeet bioassay studies the median concentration was 0.05 ppmw and four concentrations were lower. Thus, the minimum detectable concentration was 0.1 ppmw. No atrazine was detected in these samples, but it was detected in soil treated with 0.1 to 0.6 ppmw. If the concentrations of atrazine applied in the field are converted to ppm, with the assumption that initially applied atrazine was uniformly distributed throughout the top three inches of soil, one can calculate that ppm approximately equals lb ai/A. The three lowest concentrations applied in the field should have been detectable with our analytical method but were not. Because the field was plowed in the fall of 1975, atrazine was distributed through the top 10 inches of soil and further diluted. In addition, 50% or

more of the atrazine applied in 1975 would have been degraded by the time the sugarbeet crop was planted in 1976, further reducing the likelihood of detecting the lowest concentrations (3). Thus, the three lowest concentrations could not be detected.

We concluded from the greenhouse and field studies that sugarbeets are extremely sensitive to low residues of atrazine. Our results also indicate that the sugarbeet is a more sensitive analytical tool than the microcoulometer for detecting low levels of atrazine but perhaps not for precise quantification. We were unable to develop a correlation between chemical and biological analyses because the sugarbeet plant was surprisingly more sensitive to low soil residues of atrazine than suspected and gas chromatographic analysis was not sensitive enough to detect atrazine levels that the sugarbeet could. Any amount of atrazine detected by chemical assay should alert a grower to the distinct possibility of sugarbeet injury.

SUMMARY

A study was conducted to determine levels of residual atrazine in soil that injure succeeding crops of sugarbeets and to correlate the results of biological and chemical assays. Concentrations of 0.2 ppm or higher killed sugarbeets while 0.1 ppmw seriously affected sugarbeet growth in three soil types. Sugarbeets are a more sensitive analytical tool than gas-liquid chromatography with detection by microcoulometry. Because of this sensitivity to low soil residues of atrazine, correlation between biological and chemical assay was not possible. Any amount of atrazine detected by chemical assay should alert a grower to the distinct possibility of sugarbeet injury.

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