THE EFFECTS OF s-TRIAZINE AND NOTOX® 'TRIAZINE NEUTRALIZER' ON SUSCEPTIBLE CROPS

by

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ABSTRACT

Greenhouse tests were conducted to determine the effects of atrazine [2-chloro-(4-ethylamino)-6-isopropylamino)-s-triazine], simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine], and NoTox® on cotton (Gossypium hirsutum L.), wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), and alfalfa (Medicago sativa L.). Planting and application dates, soil moisture, prior adsorption of herbicide, reaction in solution, and rates were tested. Chemicals were soil-incorporated prior to planting of seeds on moist soils. Seeds were covered with dry, treated soil. Plants were thinned and sub-irrigated, and height and survival were recorded at harvest.

Preplant atrazine reduced cotton stand in the presence of NoTox. Atrazine and NoTox reduced survival of immediate and delayed cotton plantings. Cotton survival was reduced by atrazine and NoTox when preplant-irrigated and in dry soil. Wheat stands were reduced by simazine or atrazine plus NoTox with a 23-day adsorption period before planting. Alfalfa survival was reduced by an atrazine-NoTox solution and by separate chemical applications. Increased ratios of NoTox: atrazine did not improve survival of cotton and alfalfa. NoTox and simazine at the same ratios reduced stands of barley and wheat. NoTox at 8 lb/A was phytotoxic to alfalfa. Phytotoxicity of s-triazines was not reduced by NoTox in these tests.

INTRODUCTION

The s-triazine herbicides have been used extensively in selective agricultural and industrial weed control programs. Their herbicidal properties were first investigated in 1952 by J. R. Geigy Ltd. of Switzerland. The selective action and stability of the s-triazines have made them valuable tools for selective weed control in corn (Zea mays L.) and sorghum [Sorghum bicolor (L.) Moench] production in the United States.

The most significant drawback to the long-term use of the s-triazines is the persistence of their residues in soil resulting in injury to sensitive crops grown in rotation with resistant crops. Recently, research has focused on those interactions of the s-triazines with the soil environment which determine the persistence of both phytotoxic and detoxified residues in the soil.

The purpose of this study was to investigate the interactions of atrazine [2-chloro-(4-ethylamino)-6-(isopropylamino)-s-triazine], simazine [2-chloro-4,6-bis-(ethylamino)-s-triazine], and NoTox^{R} 'Triazine Neutralizer' and the affect of those interactions on cotton (Gossypium hirsutum L.), wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), and alfalfa (Medicago sativa L.).

LITERATURE REVIEW

Degradation Pathways of s-triazines

Natural or induced degradation of an herbicide may be defined as any reaction or process, the end-products of which are non-phytotoxic. In some cases, the end-products of degradation processes have not been positively identified, even though bio-assays have shown these products to be non-phytotoxic. Degradation of herbicides which produce substances more toxic than the parent material are considered to be incomplete degradation. This type of reaction was assumed by Kozlowski (1965) where toxicity of ipazine (2-chloro-4-diethylamino-6-isopropylamino-s-triazine) to pine (Pinus resinosa Ait.) seedlings was not apparent initially, but produced severe toxic symptoms 80 days after application.

The major degradation processes of the s-triazines may be grouped into three main categories. These processes are physical, biological, and non-biological reactions. The physical reactions include photodecomposition, volatilization, leaching or runoff, and adsorption. The biological processes are plant uptake and metabolism of residues by soil microorganisms. Non-biological or chemical detoxification consists of hydrolysis, N-dealkylation, and/or ring cleavage of the s-triazine molecule. The specific reactions included under these three broad categories will be considered in detail and the major environmental factors which influence them will be identified. Special emphasis will be placed on those factors which can be manipulated by man to reduce

the level of residues and crop injury which may result from the use of the s-triazine herbicides.

Physical Degradation

Photo-decomposition of s-triazines is thought to contribute little to total detoxification of the herbicide in the field. Studies by Sheets and Danielson (1960) showed that absorption of sunlight by simazine resulted in deactivation of the herbicide. Field research to determine the role of photo-decomposition has been difficult to analyze due to problems involved in separating photo-decomposition from volatilization or photolysis reactions. Photo-decomposition is optimized if the s-triazine is placed on the soil surface and minimized if it is incorporated into the soil. Low soil temperature and absorption of the herbicide onto clay particle surfaces and/or organic matter reduce the rate of photo-decomposition of the s-triazines from soil.

Most of the factors which effect photo-decomposition, similarly effect volatilization of s-triazines. Soil temperature, moisture, aeration, clay or organic matter content, and s-triazine vapor pressure determine to a great extent the movement of s-triazine molecules in the gas phase.

Leaching and run-off cannot be considered degradation reactions in the strict sense since s-triazines are not physically or chemically modified to non-phytotoxic end-products by either process. They can be considered degradation processes in the sense that the net result of leaching and run-off is removal of the herbicide from the root zone of sensitive crops. In the case of leaching, the phytotoxic residues are

still present in the soil but are unavailable for plant uptake due to the position of the residues in the soil profile. Movement of s-triazines by leaching or run-off is influenced by water solubility of the herbicide, total amount, intensity, and frequency of irrigation or rainfall, soil type and texture, and type and amount of clay and organic matter in the soil. Leaching occurs more readily in sandy soils, low in organic matter, receiving heavy, daily water applications with a highly water-soluble s-triazine. Run-off is favored over heavy or compacted soils with poor drainage, and over steeply sloping fields. The contribution of leaching and run-off to s-triazine degradation is not as important as might be expected even under irrigated conditions, primarily due to the intermediate solubility of the herbicide compared to other pesticides. Work by Upchurch and Pierce (1958) showed that herbicides must be in the soil solution prior to leaching and that the degree of adsorption onto soil particles is the primary factor influencing movement of herbicides with water through soil.

The one factor which influences nearly all degradation processes is the degree of adsorption of the s-triazine molecules onto soil particle surfaces. There is still some disagreement about the reaction mechanism involved when the s-triazine molecule becomes adsorbed onto soil colloids. Research by Weber and Coble (1968) indicated the adsorption protects some herbicides from microbial decomposition. Other work by Russel et al. (1968) showed that s-triazines must be adsorbed onto clay surfaces before detoxification by hydrolysis can occur.

The type and total amount of clay and organic matter greatly influence adsorption of s-triazines. Montmorillonite has the greatest

adsorptive capacity due to its high cation exchange capacity, large surface area, and ability to expand. Illite clay has the least adsorptive capacity due to its low cation exchange capacity and surface area. A review by Gysin and Knüsli (1960) discussed reduced loss of simazine by leaching in soils high in organic matter. Reduction of phytotoxicity of simazine in soils with high organic matter contents was observed by Rahman and Matthews (1979). Reduced loss by leaching and reduced phytotoxicity of simazine are both a function of its increased adsorption onto clay and/or organic matter.

Biological Degradation

The biological reactions responsible for degradation or removal of s-triazines from the soil are microbial metabolism and plant uptake. Microbial degradation may be a major force in the detoxification of s-triazines. Isolating microbial degradation from other degradation processes involves many of the problems associated with distinguishing photo-decomposition from volatilization of the s-triazines. Factors such as soil moisture and temperature and organic matter which increase microbial populations and activity may also favor reduced phytotoxicity due to increased adsorption or chemical hydrolysis. Disappearance curves constructed for simazine by Burnside et al. (1961) indicated almost no dissipation initially. This lag phase was thought to represent the period of time required for microbial populations to adapt to simazine as their new energy source. Work by several authors since Burnside's work seems to indicate that this lag phase is not usually apparent under field conditions. When rapid volatilization and

non-biological detoxification occur in addition to the relatively slow microbial metabolism, the lag phase is not observed. Under these conditions degradation is characterized as the first-order reaction described by Armstrong and Chesters (1967). Microbial degradation of s-triazines to non-phytotoxic metabolites consists of hydrolysis, dealkylation, ring cleavage, and conjugate formation. Knüsli et al. (1969) indicated that microbial cleavage of the s-triazine ring does not occur readily. Their work showed low levels of $^{14}CO_2$ evolution resulting from microbial degradation of ring-labeled s-triazines. This work indicated that side-chain carbons rather than ring carbons are the preferred energy sources metabolized by microorganisms.

Plant uptake is the biological process which removes s-triazines from the soil environment. Research concerning the effect of plant uptake on s-triazine persistence and phytotoxicity has been contradictory. Birk and Roadhouse (1962) showed that atrazine was more persistent in a field planted to corn than in fallow ground. Work by Sikka and Davis (1966) showed less atrazine residue in the top 15 centimeters of corn and Johnsongrass [Sorghum halepense (L.) Pers.] cropped plots than in fallow plots. Uptake and detoxification of atrazine by resistant grasses has been described by Jensen et al. (1977) as occurring by N-dealkylation, hydrolysis, and glutathione conjugation. Triazine tolerance in the grasses was found to be a function of the photosynthetic pathway and presence or absence of photorespiration. Tolerant Panicoideae grasses have C_4 (dicarboxylic acid cycle) type photosynthesis and do not undergo photorespiration. Sensitive Festucoideae grasses have Calvin Cycle photosynthesis and undergo photorespiration.

The discrepancy in residue levels between cropped and fallow ground may be partially explained by the indirect effects of plant cover on microbial activity, shading, and soil temperature, moisture, and aeration. Decomposition by biological, physical, and chemical means is generally favored by warm, moist soil conditions. Fallow ground tends to be warmer and moister, but has less biological activity than cropped ground. The interaction of these factors makes it difficult to predict the persistence of s-triazines in cropped versus fallow soil.

Non-Biological Degradation

Non-biological detoxification mechanisms consist of hydrolysis, N-dealkylation, and ring cleavage. These reactions also occur in microbial metabolism and plant and fungal detoxification. They are considered to be non-biological or chemical reactions when they occur in soil in the absence of plants or microbial activity.

Hydrolysis is the replacement of chlorine on the carbon in the 2-position with an hydroxyl group from water. Work by Hiltbold and Buchanan (1977) showed that acid hydrolysis of atrazine occurred most readily at low soil pH compared to the reaction in neutral or alkaline soil. Their work suggested that adsorption of atrazine was a prerequisite of hydrolysis, leading to the characterization of s-triazine hydrolysis as a soil-catalyzed reaction. Persistence of atrazine was observed in solution rather than when adsorbed in alkaline soils.

Hydrolysis of s-triazines occurs in plant systems as well as in soil. The major recoverable end-product of the reaction is hydroxytriazine. This process occurs in soil with atrazine, simazine, and

propazine [2-chloro-4,6-bis(isopropylamino)-s-triazine]. A distinction between acid, neutral, and alkaline hydrolysis was made by Armstrong and Chesters (1967). Alkaline hydrolysis involved direct replacement of chlorine by an hydroxyl group. Acid hydrolysis involves protonation of a ring or chain nitrogen followed by replacement of Cl by OH provided by water molecules on the clay particle surface. According to Knüsli et al. (1969) acid hydrolysis is favored under field conditions due to localized acid conditions resulting from organic matter decomposition and root or microbial exudates.

N-dealkylation of the s-triazines does not occur as readily as does hydroxylation. The process occurs in plants, animals, and by the soil fungus Aspergillus fumigatus Fres. Knusli et al. (1969) described the reaction as incomplete detoxification since the end-products remain in the soil for a long period of time and retain some phytotoxicity.

Deamination and replacement with an hydroxyl group was described by Esser et al. (1975) as the final step in detoxification of s-triazines. The end-products are cyanuric acid and ammelide which are non-phytotoxic. This reaction was considered by Kearney et al. (1965) to be an inherent part of N-dealkylation of simazine by Aspergillus fumigatus Fres. Work by Harris (1967) indicated that hydrolysis of carbon in the 2-position of the s-triazine ring occurred to a greater degree than subsequent N-dealkylation or deamination.

Ring cleavage of the s-triazine molecule consists of complete oxidation of the s-triazine heterocycle. It is of limited importance as a degradation mechanism, most of the detoxification resulting from hydrolysis and dealkylation. Cleavage via microorganism metabolism and

plant system detoxification does occur to some extent. A mechanism of ring cleavage was proposed by Gysin and Knüsli (1960) which required that hydrolysis and possibly dealkylation occur prior to ring cleavage.

Antidotes

The extensive research concerning the physical, chemical, and biological degradation of herbicides has initiated interest in how those processes can be manipulated to reduce toxic residues in agricultural soils. Antidotes can be defined as those substances that when introduced into the soil environment or plant system reduce phytotoxicity of the herbicide. There has been relatively little investigation of s-triazine antidotes, compared to the vast amount of research conducted with thiocarbamates. Triazine antidotes fall into four main categories consisting of those substances which reduce plant uptake of s-triazines, catalyze detoxification in the soil, improve plant recovery from Hill Reaction photosynthesis inhibition, and/or detoxify s-triazines in the plant system.

Adsorbents

The greatest amount of s-triazine antidote research has been accomplished in the area of altering the soil's adsorptive capacity to limit plant uptake of s-triazines. Activated charcoal is one such substance which when added to the soil adsorbs s-triazines to such an extent that they are unavailable for uptake by plants. Activated charcoal does not reduce the total amount of s-triazine residue or convert residues to non-phytotoxic products. Phytotoxicity is reduced because the residues

are made unavailable to plant systems sensitive to s-triazines. Activated charcoal ammendments can interfere with removal of residues by plant uptake. Work by Harvey (1973) showed that low rates of charcoal prevented uptake of atrazine by sorghum, corn, and Johnsongrass. Low rates of charcoal were unable to adsorb enough atrazine to prevent injury to oats (Avena sativa L.). Reduced plant uptake only becomes a problem if desorption of s-triazine from the adsorbent occurs. Desorption of atrazine from charcoal was observed in the same test when charcoal was alternately frozen and thawed, making atrazine available for plant uptake and causing injury to oats.

Activated charcoal differs in its adsorptive capacity depending on the parent material used, surface area, particle size, pore volume, and pH. The method of activating charcoal also affects its ability to adsorb organic molecules. Blair et al. (1976) showed that steam-activated charcoal was a more efficient adsorbent than chemically activated charcoal. Charcoal is selective only by its placement in the soil and has been used as a seed treatment, root dip, and banded or broadcast ammendment. The greatest limitation of its commercial use as an s-triazine antidote was the excessive rates thought to be required for adsorption of residues. Although effective rates vary with soil type, environmental factors, and crop sensitivity, as little as 56 to 84 kg/ha has been shown by Jordan and Smith (1971) to deactivate atrazine at 0.3 to 1 ppm in soil. Previous estimation of the effective rate for that specific situation was 224 kg/ha.

Other adsorbents have been investigated concerning their s-triazine antidote potential, but none have proven as effective as

activated charcoal. Weber et al. (1965) studied adsorption of some herbicides, including prometone [2,4-bis(isopropylamino)-6-methoxy-s-triazine] by a variety of adsorbents. Prometone was adsorbed onto anion exchange resins, but to much less a degree than other herbicides like 2,4-D [2,4-dichlorophenoxy)acetic acid] which behaved as organic anions in the soil. Desorption of prometone from the resin occurred when temperature was raised. Anion and cation exchange resins are of little importance as s-triazine adsorbents due to the molecular rather than ionic nature of the s-triazines.

Catalysts

There has been some field and laboratory work done in the search for catalysts of chemical hydrolysis which would accelerate the conversion of toxic s-triazines to non-phytotoxic hydroxy-s-triazines. Tipton et al. (1971) found that the component of 2-glucoside in corn responsible for detoxification of s-triazine within the plant was 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one. This cyclic hydroxamic acid catalyzed hydrolysis of simazine at low pH in solution. It was suggested that the catalyst provided more stacking surfaces which were the sites of hydrolysis of simazine.

Calcium and sodium polysulfide ions were investigated by Castelfranco and Deutsch (1962) as catalysts of simazine hydrolysis in soil. They found that between 80 and 83% of simazine present in the soil was hydrolyzed by the polysulfide ions. Oat bio-assays treated with simazine and calcium polysulfide produced fresh weights of 60 to

90% of control plants under greenhouse conditions. It is unclear why commercial use of these potential antidotes has not been developed.

Protectants

Certain substances may be considered antidotes due to their positive effects on plant vigor and ability to recover from Hill Reaction inhibition in photosynthesis. The antidotal property of CCC or chlormequat [(2-chloroethyl) trimethylammonium chloride] was studied in wheat with atrazine by Kirkland (1973). CCC in combination with atrazine reduced crop injury compared to atrazine alone. The test results were not conclusive but a possible protection mechanism by increased chlorophyll, ascorbate, and β-carotene content in plant cells was suggested.

Improved plant vigor was suggested by Ogawa and Ota (1975) to be the mechanism of simetryn [2,4-bis(ethylamino)-6-(methylthio)-s-triazine] protection of rice (Oryza sativa L.) seedlings by the fungicide 3-hydroxy-5-methyl-isoxazole.

Protection of soybeans [Glycine max (L.) Merr.] from atrazine injury by two diazosulfonates was described by Phillips and Bhagsari (1978). They found that the fungicide dexon [sodium p-(dimethylamino) benzene diazosulfonate] at 400 ppm or MBDS [sodium p-(methylamino) benzenesulfonate] at 200 ppm incorporated in soil with 0.2 ppm atrazine gave soybeans complete protection from atrazine injury. Either substance as a seed treatment was ineffective as an antidote. The mode of action suggested was detoxification within the plant and/or reduced atrazine uptake of atrazine.

NoTox

The NoTox Corporation of Lubbock, Texas has produced a new s-triazine antidote named NoTox 'Triazine Neutralizer'. Technical information published by the company reports that NoTox enhances degradation of all s-triazine herbicide residues in soil. Non-phytotoxic residues are said to be produced within 72 hours of application and incorporation of 2 parts NoTox to 1 part s-triazine residue. The directions warn that NoTox is deactivated in water in 3 hours which limits the preparation and storage time of NoTox solution prior to its application to soil. According to the same labeling, NoTox is deactivated within 5 days of application and soil incorporation. The proposed mode of action of NoTox with an s-triazine molecule is removal of chlorine from the carbon in the 2-position followed by cleavage of the carbon-nitrogen double bonds of the ring. NoTox is an activated oxygen in a powder formulation, with an LD_{50} of 2250 mg/kg in rats.

METHODS AND MATERIALS

Ten experiments were conducted at a University of Arizona's Campbell Avenue greenhouse, between December 7, 1979 and June 25, 1980. The experiments determined survival and growth responses of s-triazine-sensitive crops when grown in soil treated with atrazine or simazine and NoTox 'Triazine Neutralizer'.

The commercial formulations of herbicides used were Aatrex® 80% WP and Princep® 80% WP. The varieties of s-triazine sensitive crops grown were 'Deltapine 61' or '70' cotton, 'Lew' alfalfa, 'Arivat' barley, and 'Mexicale' durum wheat.

Common Procedures

The following procedures are common to each of the tests conducted. Any deviation in procedures is noted for the test to which it applies. Plants were grown in 400 cc plastic pots containing sandy loam (60% sand, 25% silt, 15% clay) soil with 1% organic matter. The soil was taken from the field, air-dried, and passed through a 635 mm mesh screen. The bottom of each pot was perforated and the holes were plugged with cotton to allow water drainage and uptake from surface and sub-irrigations. A few grains of ammonium-phosphate fertilizer were placed on the soil surface for each pot.

The herbicides and NoTox were sprayed over the pots using a backpack sprayer with a single boom fitted with an 8003 nozzle tip at 2.8 kg/cm^2 pressure. Desired rates of each chemical were mixed with an

amount of water equivalent to 375 liters of water per hectare. All applications, except those for preemergence and postemergence treatments in the time of application test for cotton, were preplant applications which were soil incorporated by shaking in a closed container for approximately 30 seconds.

All but 0.6 to 1.3 cm of treated soil was returned to each pot and surface irrigated. Enough water was applied to the soil surface to reach the bottom of the pot. Seeds were placed on the moist soil and covered with the remaining 0.6 to 1.3 cm of dry, treated soil. All pots were placed on a baking tin and loosely covered with plastic sheeting to prevent desiccation of germinating seeds. The plastic sheet was removed as plants emerged. Each tray of pots was sub-irrigated as needed throughout the period of the test. Treatments were replicated three times.

Emerging cotton plants were thinned to four per pot, producing a total of 12 plants per treatment. Alfalfa, barley, and wheat were thinned to 10 plants per pot, producing a total of 30 plants per treatment for each of those crops. Each test was harvested when phytotoxic symptoms were apparent. The time intervals between planting and harvest are noted for each test. Harvest consisted of counting the number of green, erect plants and measuring maximum foliar growth for each plant. Treatment averages were based on averaging replication averages. Cotton, alfalfa, and wheat plants were counted as surviving only if all foliage was erect. Barley plants were considered alive if at least one leaf blade of the plant remained erect at the time of harvest. Cotton height was measured from the soil surface to the bottom of the leaf blade for

the tallest leaf. Alfalfa, barley, and wheat plant heights were measured from soil surface to leaf tip of the longest leaf blade.

Time of Atrazine and NoTox Application in Cotton

Preplant and preemergence soil applications of atrazine at 1.1 kg/ha and NoTox at 2.2 kg/ha were made on December 7, 1979. Preplant treatments were soil incorporated to simulate disking of herbicide and neutralizer into the soil. Incorporation was accomplished by tumbling the soil on plastic sheeting immediately prior to planting 'Deltapine 70' cotton. Preemergence applications of atrazine and NoTox were made immediately after planting on December 7. Postemergence applications were made 14 days after planting without sub-irrigation between planting and herbicide or neutralizer application. Growth and survival were recorded 20 days after planting.

Ratio of NoTox to Atrazine in Cotton

Preplant applications of atrazine at 0 and 1.1 kg/ha and NoTox at 0, 2.2, 4.5, and 9 kg/ha were made on January 8, 1980. Atrazine, NoTox, and fertilizer were soil incorporated immediately prior to planting 'Deltapine 61' cotton. Seedling growth and survival were recorded 24 days after treatment and planting.

Low Rates of Atrazine and NoTox in Alfalfa

Preplant combinations of atrazine at 0, 0.14, 0.3, and 0.6 kg/ha and NoTox at 0, 0.3, 0.6, and 1.1 kg/ha were applied on June 10. Herbicide, neutralizer, and fertilizer were soil incorporated immediately and seed were planted 4 days later. Pots were not sub-irrigated between

treatment and planting. Plant survival and growth were recorded 11 days after planting.

Ratio of NoTox to Atrazine in Alfalfa

Preplant applications of atrazine at 0 and 1.1 kg/ha and NoTox at 0, 2.2, 4.5, and 9 kg/ha followed by soil incorporation were made on April 2. Alfalfa was planted immediately after incorporation and plant survival and growth were recorded 15 days later.

Ratio of NoTox to Simazine in Barley

Preplant application and incorporation of simazine at 0 and 1.1 kg/ha and NoTox at 0, 2.2, 4.5, and 9 kg/ha were made on April 2. Barley was planted immediately after incorporation and survival and growth were recorded 19 days later. Height measurements were made for collapsed as well as erect foliage.

Ratio of NoTox to Simazine in Wheat

Preplant application and incorporation of simazine at 0 and 1.1 kg/ha and NoTox at 0, 2.2, 4.5, and 9 kg/ha were made on April 2. Wheat was planted immediately after incorporation and plant survival and growth were recorded 19 days later.

Preplant Irrigation Following Atrazine and NoTox in Cotton

Atrazine at 0 and 1.1 kg/ha and NoTox at 0 and 2.2 kg/ha were applied and incorporated on January 18. Half of the treatments received surface irrigation immediately after incorporation. The other half were

left dry. Seven days later the soil was surface irrigated and cotton was planted in all treatments. Plant survival and growth were recorded 19 days after planting.

Delayed Planting with Atrazine and NoTox in Cotton

Atrazine at 0 and 1.1 kg/ha and NoTox at 0 and 2.2 kg/ha were applied and incorporated on January 11. 'Deltapine 61' cotton was planted immediately in half of the treatments. The other half were planted 7 days later. Plant survival and top growth were recorded 21 days after planting of each treatment.

Delayed NoTox with Atrazine and Simazine in Wheat

Atrazine or simazine at 0 and 1.1 kg/ha were applied and incorporated on April 2. In six of eight treatments NoTox at 0 and 2.2 kg/ha was also applied and incorporated on April 2. In the other two treatments, NoTox at 2.2 kg/ha was applied and incorporated on April 25, which was 23 days after atrazine application. Wheat was planted on May 2 for all treatments which provided a 30-day-period between NoTox-herbicide applications and planting in six treatments, and a 7-day-period between NoTox application and planting in two treatments. Survival and growth of seedlings were recorded 17 days after planting. Those plants with at least one erect leaf were included in the survival figure for this test.

Tank-Mixed NoTox and Atrazine in Alfalfa

Atrazine at 0 and 0.6 kg/ha and NoTox at 0 and 1.1 kg/ha were applied to the soil consecutively or as a combined, tank-mixed solution

and were incorporated on June 10. Alfalfa was planted 4 days later and seedling growth and survival were recorded 11 days after planting. Heights of collapsed plants were not recorded.

RESULTS AND DISCUSSION

In the following tests, percent survival and maximum top growth of cotton, wheat, barley, and alfalfa were used as indicators of the presence or absence of phytotoxic residues of simazine or atrazine in the soil.

Time of Atrazine and NoTox Application in Cotton

Table 1 lists times of application and cotton responses to each treatment. Plant survival was reduced by 83 to 100% of the untreated check plants by preplant application of atrazine at 1.1 kg/ha regardless of the time of application of NoTox at 2.2 kg/ha (Table 1). Stands were not reduced when atrazine was applied preemergence regardless of the time of application of NoTox. Postemergence atrazine and NoTox did not reduce stands. However, preplant NoTox followed by postemergence atrazine significantly reduced survival of cotton. The complete stands of cotton in the presence of atrazine and NoTox may have been due to placement of the herbicide in the soil such that no contact was made between developing roots and atrazine rather than to any reduced atrazine phytotoxicity by NoTox. All pots were sub-irrigated following treatment which would help keep atrazine from moving down into the root zone. It is also possible that some volatilization of atrazine may have occurred since preemergence and postemergence applications at atrazine were not soil incorporated.

Table 1. Cotton seedling response to preplant, preemergence, and postemergence applications of atrazine and NoTox.

Treatment		Response	
Atrazine (2.2 kg/ha)	NoTox (2.2 kg/ha)	Survival[a] (%)	Height[a] (cm)
untreated check		100 a	12 a
Preplant	Preplant	17 cd	2 c
Preplant	Preemergence	17 cd	3 bc
Preplant	Postemergence	0 d	0 c
Preemergence	Preemergence	100 a	8 ab
Preemergence	Postemergence	75 ab	10 a
Postemergence	Postemergence	100 a	9 a
Postemergence	Preplant	50 bc	5 b

[a]Values within a column followed by the same letter are not significantly different.

Ratio of NoTox to Atrazine in Cotton

The ratio of NoTox to s-triazine residue required for detoxification recommended in NoTox Corporation technical information is 2:1. This test examined plant response to the recommended dose of antidote and the response to 2 and 4 times the recommended dosage. Atrazine alone reduced plant survival by 92% of the untreated checks (Table 2). No plants survived treatment with atrazine at 1.1 kg/ha plus 2.2 kg/ha NoTox. The response of plants treated with 1.1 kg/ha atrazine plus NoTox at 4.5 or 9 kg/ha was the same as that of plants treated with atrazine alone. Top growth of plants treated with 1.1 kg/ha atrazine plus NoTox at 2.2, 4.5, and 9 kg/ha was reduced by 100, 40, and 50% respectively. Response trends in this test were not in agreement with the results of work by Owens and Eaton (1980) in which a 10:1 ratio of NoTox to atrazine produced a 15 to 30% increase in yield in a wheat bio-assay.

Low Rates of Atrazine and NoTox in Alfalfa

The technical data published by the NoTox Corporation does not indicate a threshold concentration of s-triazine residue over which NoTox is ineffective as a residue-neutralizer. However, it was thought that atrazine at 1.1 kg/ha might overload the neutralizing capacity of NoTox. And, 1.1 kg/ha is probably excessive in terms of the amount of residue that may be expected to persist in soil after one season of cropping with average field conditions. This test maintained the recommended 2:1 NoTox-s-triazine ratio since improved protection was not apparent in tests where the ratio was increased to 8:1. The required

Table 2. Cotton responses to preplant applications of atrazine and NoTox.

Treatment		Response	
Atrazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		100 a	10 a
0	2.2	100 a	10 a
0	9	100 a	11 a
1.1	0	8 b	7 b
1.1	2.2	0 b	0 b
1.1	4.5	8 b	6 b
1.1	9	8 b	5 b

[a]Values within a column followed by the same letter are not significantly different.

reaction time of 72 hours in soil is also surpassed in this test. Four days of reaction time were allowed prior to planting alfalfa.

Alfalfa survival was reduced by 62, 97, and 100% of untreated checks when treated with atrazine alone at 0.14, 0.3, and 0.6 kg/ha respectively (Table 3). NoTox plus atrazine at the rates noted and at a ratio of 2 NoTox:1 atrazine, reduced alfalfa survival by 86, 100, and 100% of untreated checks, respectively. A comparison of plant survival when treated with atrazine plus NoTox, compared to plants treated with atrazine alone shows a 63 to 100% decrease in survival of plants receiving the atrazine plus NoTox treatments. The decreased survival in the presence of NoTox and atrazine is difficult to explain since there is no treatment with NoTox alone which might show a toxic reaction of alfalfa to low rates of NoTox alone. These conditions were examined with alfalfa at higher NoTox rates in the following test.

Ratio of NoTox to Atrazine in Alfalfa

Atrazine alone reduced survival by 100% (Table 4). NoTox alone at 9 kg/ha reduced survival and growth of plants compared to the untreated plants. NoTox alone at 4.5 kg/ha did not affect either survival or growth of alfalfa.

Survival responses to atrazine plus NoTox at all combined rates were the same as responses in the presence of atrazine alone. These treatments resulted in a complete loss of all alfalfa seedlings.

Ratio of NoTox to Simazine in Barley

Survival of barley treated with simazine alone at 1.1 kg/ha was reduced by 87% (Table 5). Simazine combined with NoTox at 2.2 and 4.5

Table 3. Alfalfa responses to preplant applications of low rates of atrazine and NoTox.

Treatment		Response	
Atrazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		97 a	5 a
0.14	0	37 b	4 ab
0.14	0.3	13 c	3 bc
0.3	0	3 c	2 c
0.3	0.6	0 c	0 d
0.6	0	0 c	0 d
0.6	1.1	0 c	0 d

[a]Values followed by the same letter within a column are not significantly different.

Table 4. Alfalfa responses to preplant applications of atrazine and NoTox.

Treatment		Response	
Atrazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		87 a	6 b
0	2.2	90 a	6 b
0	4.5	83 a	7 a
0	9	47 b	5 c
1.1	0	0 c	0 d
1.1	2.2	0 c	0 d
1.1	4.5	0 c	0 d
1.1	9	0 c	0 d

[a]Values followed by the same letter within a column are not significantly different.

Table 5. Barley responses to preplant applications of simazine and NoTox.

Treatment		Response	
Simazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		100 a	22 a
0	2.2	77 a	17 bc
0	4.5	97 a	20 ab
0	9	83 a	20 ab
1.1	0	13 b	13 d
1.1	2.2	13 b	15 cd
1.1	4.5	13 b	12 d
1.1	9	10 b	13 d

[a]Values followed by the same letter within a column are not significantly different.

kg/ha resulted in the same survival rate as did simazine alone at 1.1 kg/ha. Simazine at 1.1 kg/ha plus NoTox at 9 kg/ha reduced survival by 90%. Plant stunting in the presence of simazine at 1.1 kg/ha plus NoTox at 2.2, 4.5, and 9 kg/ha ranged from 32 to 45% of untreated check plants.

Ratio of NoTox to Simazine in Wheat

Wheat was less sensitive to NoTox alone at 4.5 and 9 kg/ha than was alfalfa. No stand loss or stunting was observed in those treatments (Table 6). The stand reduction at 2.2 kg/ha NoTox was probably due to experimental error. The survival of wheat with simazine alone was not significantly different than survival with 1.1 kg/ha simazine plus NoTox at 2.2, 4.5, and 9 kg/ha.

Preplant Irrigation Following Atrazine and NoTox in Cotton

Owens and Eaton (1980) stated that "the chemical reaction between the s-triazine residues and the NoTox must occur in the water solution of the field." If the mechanism of detoxification is removal of chlorine from the carbon in the 2-position plus cleavage of carbon-nitrogen double bonds, as stated in the NoTox Corporation's technical information, this reaction must be considered a chemical hydroxylation of the s-triazine molecule. Extensive work by many authors, specifically Hiltbold and Buchanan (1977) has shown that hydrolysis or hydroxylation is accelerated, rather than inhibited by the addition of soil to solutions of s-triazines. Their work indicates that surfaces of soil colloids provide the site of hydrolysis of atrazine, and that persistence of atrazine is extended if atrazine is in the solution phase rather than in the adsorbed phase in soil.

Table 6. Wheat responses to preplant applications of simazine and NoTox.

Treatment		Response	
Simazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		100 a	22 abc
0	2.2	50 b	20 bc
0	4.5	100 a	25 a
0	9	90 a	23 ab
1.1	0	7 c	20 bc
1.1	2.2	10 c	20 bc
1.1	4.5	20 bc	19 cd
1.1	9	0 c	16 d

[a]Values followed by the same letter within a column are not significantly different.

All of the tests already discussed have involved interaction of simazine or atrazine with NoTox when incorporated into dry soil followed by surface irrigation immediately prior to planting. If water is required for the neutralizing reaction between s-triazine and NoTox, as was suggested in the text of Owens and Eaton's paper, then there would not have been enough time for detoxification between wetting the treated soil and planting in those tests. The phytotoxicity of atrazine or simazine plus NoTox in the previous tests (excluding the test of low rates of atrazine with alfalfa), may perhaps be due to exposure of seedlings to unreacted herbicide prior to the 72 hours required for completion of detoxification by NoTox. The next experiment tested this idea.

The test was designed to allow increased time for atrazine and NoTox to react in dry and moist soil prior to planting. Under pre-irrigated conditions, the presence of 1.1 kg/ha atrazine with and without 2.2 kg/ha NoTox, significantly reduced the stand and stunted plants compared to untreated plants and plants with NoTox alone (Table 7). The same responses were observed when plants were not immediately irrigated after herbicide and/or NoTox application.

Delayed Planting of Cotton with Atrazine and NoTox

This test was designed to allow an extended time for atrazine and NoTox to interact in soil prior to planting. The soil remained dry between treatment and the planting irrigation. All plants died when treated with atrazine and NoTox regardless of the time of planting

Table 7. Cotton responses to atrazine and NoTox with and without pre-irrigation.

Treatment		Response	
Atrazine	NoTox	Survival[a] (%)	Height[a] (cm)
With Pre-Irrigation			
untreated check		100 a	11 a
0	2.2 kg/ha	100 a	11 a
1.1 kg/ha	0	8 b	5 b
1.1 kg/ha	2.2 kg/ha	0 c	0 b
Without Pre-Irrigation			
untreated check		100 a	11 a
0	2.2 kg/ha	100 a	10 a
1.1 kg/ha	0	0 c	0 b
1.1 kg/ha	2.2 kg/ha	8 b	6 b

[a]Values followed by the same letter within a column are not significantly different.

(Table 8). Atrazine alone at 1.1 kg/ha reduced plant survival by 83% of untreated plants at both planting dates.

Delayed NoTox Application with Atrazine and Simazine in Wheat

It is possible that NoTox is unable to attack and neutralize s-triazine molecules unless they are first adsorbed onto soil particles. The idea that adsorption may be a prerequisite for hydrolysis of an herbicide molecule was suggested by Hiltbold and Buchanan (1977). In this test, a 23-day-delay between herbicide incorporation and NoTox application and incorporation was allowed for adsorption of herbicide onto soil. Survival of wheat was significantly reduced in the presence of atrazine or simazine, regardless of the presence or time of application of NoTox (Table 9).

Tank-Mixed Atrazine and NoTox with Alfalfa

Stand reduction and plant stunting were significant in the presence of 0.6 kg/ha atrazine with and without 1.1 kg/ha NoTox. There was no significant difference in response to atrazine and NoTox when applied as a pre-mixed solution or when applied separately (Table 10). This test was primarily of academic interest. If the results of the test had shown that NoTox neutralized atrazine in solution, prior to its application, there would be no practical application of the reaction since the triazine to be neutralized under field conditions would already be in the soil.

Table 8. Cotton responses to atrazine and NoTox with a 7-day-interval between preplant application and planting.

Treatment			Response			
Atrazine (kg/ha)	NoTox	Planted on:	Survival[a] (%)		Height[a]	
Jan 11			Jan 11	Jan 18	Jan 11	Jan 18
untreated check			100 a	100 a	11 a	10 a
0	2.2		100 a	100 a	12 a	10 a
1.1	0		17 b	17 b	4 b	3 bc
1.1	2.2		0 c	0 c	0 c	0 c

[a]Values followed by the same letter are not significantly different.

Table 9. Wheat responses to atrazine and simazine with immediate and delayed NoTox applications.

Treatment		Response	
s-Triazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		73 b	22 a
0	2.2	100 a	21 a
Atrazine 1.1	0	0 c	0 b
Atrazine 1.1	2.2	0 c	0 b
Atrazine 1.1	2.2[b]	0 c	0 b
Simazine 1.1	0	0 c	0 b
Simazine 1.1	2.2	3 c	5 b
Simazine 1.1	2.2[b]	0 c	0 b

[a]Values followed by the same letter within a column are not significantly different.

[b]NoTox applied and incorporated 23 days after herbicide application and incorporation.

Table 10. Alfalfa responses to atrazine and NoTox, applied consecutively and as a pre-mixed spray.

Treatment		Response	
Atrazine (kg/ha)	NoTox	Survival[a] (%)	Height[a] (cm)
untreated check		93 a	5 a
0	1.1	100 a	5 a
0.6	0	0 b	0 b
0.6	1.1	0 b	0 b
0.6	1.1 tank-mixed	3 b	1 b

[a]Values followed by the same letter within a column are not significantly different.

SUMMARY

In general, NoTox neither increased nor decreased phytotoxicity of s-triazines compared to the effect of herbicide alone on the plants. In all tests, seedlings emerged normally. NoTox did not protect plants from s-triazine injury in any of the tests.

There were two situations in which the combined herbicide and antidote treatments were more phytotoxic than the herbicide treatment. These cases involved alfalfa and cotton seedlings. Alfalfa survival was 37% when treated with 0.14 kg/ha atrazine and only 13% when treated with 0.14 kg/ha atrazine plus 0.3 kg/ha NoTox. In the delayed planting test, 17% of cotton plants survived when treated with 1.1 kg/ha atrazine. A complete stand loss resulted when soil was treated with 1.1 kg/ha atrazine plus 2.2 kg/ha NoTox at both immediate and delayed planting dates.

One case of NoTox phytotoxicity was observed. Only 47% of alfalfa plants survived applications of 9 kg/ha NoTox compared to 87% survival of untreated check plants.

Rates and ratios of NoTox with atrazine and simazine, soil moisture, time of planting and application, and prior adsorption of s-triazine in soil have been examined as to their effect on s-triazine neutralization by NoTox. The results of the bio-assays indicated that residues of atrazine and simazine remained phytotoxic in soil regardless of the presence of NoTox.

More field and laboratory research is needed before consistent enhanced detoxification of s-triazine herbicides by NoTox can be claimed without qualification.

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