PHYSIOLOGICAL STUDIES WITH THE EXPERIMENTAL HERBICIDE ISOURON

by

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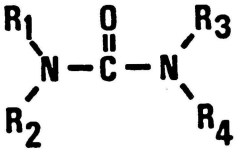
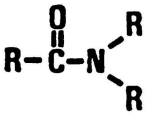
INTRODUCTION AND OBJECTIVES

The substituted urea herbicides are a relatively large and diverse group of chemicals. Since the discovery of their phytotoxic effects in the early 1950s, this class of herbicides has taken an important place in agricultural weed control programs and in laboratory based photosynthetic research. As a group they have two important features in common: 1) a urea backbone  and 2) the ability to

effectively inhibit photosynthetic electron flow from photosystem II to photosystem I. Diversity within this group arises from various substituents on the nitrogens of the urea skeleton. It is believed that the activity of these compounds as photosynthetic inhibitors arises from the lactam moiety,  (28, 30). The actual degree of

phytotoxicity and the selective activity of the ureas in plants is associated with the substituents present at R_1, R_2, R_3 and R_4 of the urea molecule. Most of the urea herbicides which are commercially available are substituted phenylureas (R_2 = phenyl ring, R_1 = H). Much academic and commercial research has been conducted with the substituted phenylureas, and their mode of action is fairly well established.

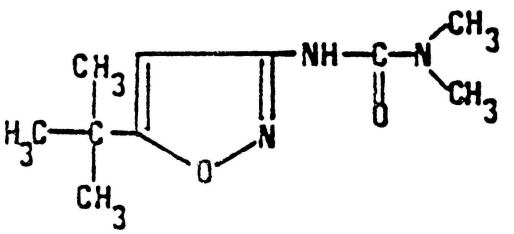
Recently a new class of substituted ureas was introduced, in this and other countries, for testing as potentially useful and marketable herbicides. In this new class of herbicides, R_2 is a nitrogen and oxygen containing heterocyclic ring (isoxazolyl ring) with substituents on the number 4 carbon of this ring. One such herbicide, isouron (N'-(5-(1,1-dimethylethyl)-3-isoxazolyl)-N,N-dimethylurea), was discovered to have relatively good herbicidal properties, i.e. low toxicity to mammals and high toxicity to plants at fairly low rates, and its commercial development was being pursued by Eli Lilly and Company.

The laboratory studies reported in this paper were conducted to investigate the effect of the new herbicide isouron and two of its metabolic byproducts found in plants on four metabolic processes of enzymatically isolated soybean leaf cells. These processes were photosynthesis, protein synthesis, lipid synthesis and RNA synthesis. Also, the I_{50} (herbicide concentration at which a metabolic process is inhibited by 50%) value was determined for photosynthesis as inhibition of photosynthesis appeared to be the primary mode of action of isouron. Finally, the influence of isouron on the breakdown of chlorophyll was investigated, as several researchers have indicated that chlorophyll breakdown resulting from the production of free radicals induced by some urea herbicides may be a secondary mode of phytotoxic activity of the ureas (37, 47).

Followup greenhouse studies were also conducted. The intent of these studies was to evaluate the protective activity of four commercial antidotes on two varieties ('Tyler' and 'McNair 1003') of wheat (Triticum

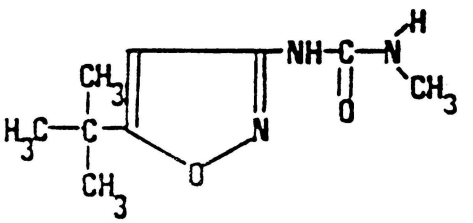
aestivum L.) and two varieties ('XL72AA' and 'XL67') of corn (Zea mays L.) from the phytotoxic effects of isouron. The antidotes used were 1,8 naphthalic anhydride (NA), a seed applied antidote shown previously to protect grass crops (mainly corn, sorghum (Sorghum bicolor Moench) and rice (Oryza sativa L.)) from thiocarbamate and acetanilide injury, N,N-diallyl-2,2-dichloroacetamide (R-25788), a soil applied, preplant incorporated antidote which protects corn from thiocarbamate and acetanilide injury, α-((cyanomethoxy) imino) benzene acetonitrile (CGA-43089) which is used as a seed dressing on sorghum seeds to prevent acetanilide injury, and α-((1,3-dioxolan-2-yl-methoxy) imino) benzene acetonitrile (CGA-92194), an analog of CGA-43089, also used to prevent acetanilide injury to sorghum.

At present there are no known effective antidotes against photosynthesis inhibiting herbicides. Screening of this type may help in establishing a basis for work toward such antidotes by revealing compounds with potential for protection or by ruling out structures which show no effect or show synergism with the photosynthesis inhibiting herbicides.



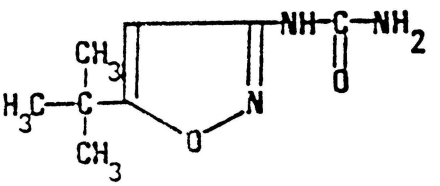
N-[5-(1,1-dimethylethyl)-3-isoxazolyl]-N,N-dimethylurea

Isouron



N-[5-(1,1-dimethylethyl)-3-isoxazolyl]-N-methylurea

Metabolite 1



N-[5-(1,1-dimethylethyl)-3-isoxazolyl]-urea

Metabolite 2

Figure I-1. Chemical structures of isouron and its two plant metabolites.

LITERATURE REVIEW

Urea Herbicides. In 1951, Bucha and Todd (4) introduced a new herbicide 3-(p-chlorophenyl)-1,1-dimethylurea (CMU or monuron) and discussed its phytotoxic effects as a postemergence spray to several annual and perennial grass weeds and crops. A more complete discussion of the phytotoxic effects of monuron was presented in a paper by Christoph and Fisk in 1954 (5). They described stunting of plants grown in treated soil, as well as weakened stems, chlorosis, progressive leaf tip necrosis and eventual plant death. They suggested that the photosynthetic processes may be impaired by monuron. In 1956, using suspensions of isolated chloroplasts and reduction of a dye, Janus green B, as a measure of photosynthetic rate, Cooke (3) hypothesized that the mode of action of the urea herbicides was inhibition of photosynthesis. Subsequent research with the ureas verified these early studies, indicating photosynthesis as the main site of action, although the exact mechanisms remained unknown (8, 23, 36, 41, 48, 50).

Since these early days of the urea herbicides, this group of compounds has grown in number and importance. Presently there are some 21 urea herbicides registered for use in the United States. Their primary use is in the preemergence control of annual grasses and broadleaf weeds in tolerant crops. Postemergence applications are also used in cropland areas, although crop tolerance to such applications is generally low and they must be made in a postemergence directed manner to avoid foliar crop contact. Broad spectrum weed control in

noncropland areas can also be attained with urea herbicides. The importance of the ureas is not limited to the field, however. Soon after their discovery it was found that members of this group, especially diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), could be used to accurately and selectively block photosynthetic electron flow from photosystem II (PS II) to photosystem I (PS I) in laboratory studies with chloroplasts. This allowed separation of the two photosystems and thus permitted more detailed and specific work toward defining the functional variation between PS II and PS I which eventually helped elucidate the mechanisms of photosynthetic activity. Today diuron (DCMU) is used in combination with other inhibitors and electron donors to examine specific reactions in photosynthetic electron flow.

Absorption and Translocation. The urea herbicides are mainly root absorbed compounds which move apoplastically to the shoots and stems of plants growing in treated soil.

Absorption of these herbicides into roots from soil or nutrient solution is a passive process (9, 16, 18) occurring most likely by diffusion, although a higher concentration of herbicide can exist in the root than in the surrounding soil solution due to the tendency of urea herbicides to bind strongly to cellulose molecules and other structural polysaccharides (13).

Early work on translocation of ureas was first done with monuron (5, 24). This work revealed development of leaf symptoms when only the roots of the plant had been exposed to the herbicide. Thus it was hypothesized that the herbicide moved in the xylem. This was partially

confirmed in 1955 by the work of Fang et al. (10) using radiolabeled monuron. This group observed rapid movement of ^{14}C monuron from treated roots to the shoots of bean plants. Also, it was observed that movement from a treated leaf to other plant parts was extremely limited, i.e. movement in the assimilate stream of ureas is almost nonexistent. Later work with ^{14}C-chloroxuron (3-(p-(p-chlorophenoxy) phenyl)-1,1-dimethylurea) (13) revealed that apoplastic movement was basically the main means of transport of this herbicide in plants, and that the rate of movement was influenced by temperature and moisture, i.e. factors that affect transpiration. Apoplastic movement is believed to be the major route of urea herbicide translocation.

Metabolism. Much of the work in the area of urea metabolism has been done with phenylureas, with only limited information available for heterocyclic or saturated hydrocarbon rings as substituents on the urea molecule. N-demethylation appears to be the primary metabolic step in degradation of urea herbicides in plants. In most cases the monomethylurea derivative is less toxic than the parent compound (2, 39). A second demethylation often occurs, resulting in a urea derivative which is even less toxic, often exhibiting little or no effect as a herbicide. N-dealkylation or N-dealkoxylation of alkyl or alkoxy ureas follows a similar pattern (2, 20). Work done with isouron indicates that these two metabolic steps are most important (19).

Ring hydroxylation is considered a second major pathway of metabolic detoxication. Hydroxylation is often followed by formation of O-glycosides, a common detoxication step in many living systems (17, 20,

21). Another step common to phenylurea metabolism is the oxidation of ring substituents. At present, oxidation of halogen substituents directly has not been shown. Substituents such as alkyl or alkoxy groups attached to rings in such herbicides as chlorotoluron (3-(3-chloro-4-methylphenyl)-1,1-dimethylurea), metoxuron (N'-(3-chloro-4-methoxyphenyl)-N,N-dimethylurea) and difenoxuron (N'-4-(4-methoxyphenyl-N,N-dimethylurea) have been shown to be susceptible to oxidation. Such oxidations may proceed only to the hydroxylated intermediate or may continue to form the corresponding acid. Hydroxylation reactions may be an intermediate step in N-demethylation and N-dealkylation reactions (12, 17, 19, 20), however direct evidence for such reactions is not available. The above mentioned oxidation reactions are thought to be catalyzed by the ubiquitous group of enzymes known as mixed function oxidases, thought to be associated with the microsomal fraction of the plant cell. Frear (11) recognized this in cotton (Gossypium hirsutum L.) leaf metabolism of monuron, in which the enzymes necessary for N-demethylation were found to be associated with microsomes and were dependent on O_2 and NADPH (or NADH) for activity. Frear (12) also first reported the isolation of N-hydroxy-methyl and O-glycosides of monuron in cotton leaves.

Another minor pathway of degradation of phenylureas is plants is that of aniline formation (20). Such formation only occurs after dealkylation reactions and consists of a hydrolysis of the N-dealkylated ureas to their corresponding anilines with the release of CO_2 and NH_3. Reports of this degradation pathway are not well confirmed. It does

not appear that the urease enzyme systems play any role in these reactions. At present N-demethylation to the monomethylurea and urea derivatives appears to be the major pathway for isouron metabolism. Formation of methyl-hydroxy intermediates and O-glycosides has also been reported for this herbicide (19).

Mode of Action.

Introduction. The mode of action of the urea herbicides has been the subject of intensive study since the initial report of the toxicity of monuron to grasses in 1951 (4). Cooke's (6) work in 1954 with isolated chloroplasts and monuron suggested photosynthesis as the primary site of action. These results were soon reinforced with reports of light induced toxicity to stems and leaves of urea herbicide treated plants, while the plant roots remained relatively unaffected (24, 25, 26, 27). In 1960, Minshall (27) reported a marked suppression of non-water soluble carbohydrate levels in detached bean leaves treated with monuron and diuron. Thus he suggested that photosynthetic CO_2 fixation was being inhibited by a "poisoning" action of these two urea herbicides, and that the importance of light in the development of phytotoxicity indicated that the herbicide is active at the water hydrolysis step or Hill reaction of photosynthesis. These early hypotheses were not entirely wrong. Electron flow from the splitting of H_2O in the Hill reaction is inhibited by the urea herbicides, however the inhibition takes place on the reducing side of PS II, as revealed by careful observation and improved empirical techniques. Specifics on the exact mode of action of ureas will be addressed later in this section.

Structure Activity Relations. It was recognized very soon after the discovery of the phytotoxicity of the ureas that these compounds had varying degrees of activity, i.e. specific molecular configurations of each molecule influenced its phytotoxic capabilities. In 1966, Hansch (14) addressed this phenomenon. His work established a link between the nature of the substituent side chains on the urea backbone and activity. A correlation was realized between the lipophilic characteristics of the side chains and the degree of phytotoxicity; increasing lipophilicity resulted in greater toxic action of the substituted ureas. The nature of these structure activity relations of the urea herbicides was investigated further in 1969 in two papers, by Hansch (15) and Moreland (29). The ubiquitous toxicity of the lactam moiety was recognized by both researchers. Hansch proposed that this moiety could interact at many sites by binding with amide linkages of proteins, the nitrogen atom of the herbicide molecule interacting with the carbon of the protein amide and the carbon of the herbicide with the nitrogen of the protein amide. Such interactions are not uncommon in living systems. Interactions of this kind have been implicated as important steps in enzyme catalyzed reactions. Hansch proposed that this interaction was necessary for initial binding of the herbicide to an active site and that further molecular interactions could then take place, i.e. hydrogen bonding, that would further enhance herbicide-active site binding. With this initial binding intact, further interactions of the hydrophobic side chains would allow specific binding of the herbicide into a suitable active

site, thus giving an explanation of the selective binding mechanism which would have to occur to allow for the observed differential susceptibility of plants to the ureas. Moreland elaborated on this concept by suggesting that the inhibitor must possess properties that will allow penetrations to the active site as well as precise spacial configurations to allow appropriate binding to the active site. He suggested properties such as partitioning characteristics (lipophilic vs. hydrophilic), steric relations, possession of critical atomic charge, and cis-trans configurations as critical to herbicidal activity.

Characteristics of the Binding Site. With a fairly sound understanding of the specifics for herbicidal activity in hand, i.e. the lactam moiety with hydrophobic side chain of the nitrogen molecule and the presence of atomic substitutions on this side chain influencing activity, characterization of the urea binding site was begun. In 1977, Tischer and Strotman (42) discovered a competitive binding relationship between many of the known photosynthesis inhibiting herbicides. Using radiolabeled herbicides, they revealed that the ureas, triazines, triazinones, pyridazinones and carbamates all exhibited competition for a similar binding site in the chloroplast. Kinetic studies were done and it was revealed that there was approximately one binding site per 300-500 chlorophyll molecules, or approximately one binding site per PS II reaction center complex. Also the energy of binding for metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one) was determined to be approximately -50 kJ/mol (-12 kcal/mol). This number was reproduced in another set of experiments (43). Such a binding energy is too

low for a covalent interaction and too high for a simple hydrogen bonding interaction. They also found a correlation between inhibitor binding and interruption of electron transport from PS II to PS I.

In 1976, Renger et al. (34) observed the inhibitory effects of trypsin treatments on photosynthetic electron flow between photosystems in isolated spinach (Spinacia oleracea L.) chloroplasts. These researchers surmised that an important proteinaceous component of the electron chain was altered by the trypsin which resulted in disrupted electron transport. Also in 1976 Renger (33) reported a decrease in diuron binding through trypsin treatment of isolated chloroplasts. He hypothesized that a proteinaceous component present in chloroplast membranes known as the Q-B protein shield was broken down by the trypsin treatment. This Q-B protein is believed to interact allosterically with components of the electron chain, i.e. the electron carriers Q and B, to facilitate proper electron flow through the chain. The breakdown in this protein caused by trypsin digestion resulted in decreased diuron binding as well as decreased photosynthetic electron flow. Use of specific water soluble electron acceptors, i.e. $K_3(Fe(CN)_6)$, could circumvent this trypsin induced electron blockage. Therefore, he proposed that trypsin induced breakdown of the Q-B protein was exposing the electron transport system to potassium ferricyanide allowing this to carry electrons artificially to PS I around the trypsin induced block. Thus he hypothesized the Q-B protein must be important in the transfer of electrons from PS II to PS I and that this may be the binding site of diuron.

In 1979, Croze et al. (7) reinforced Renger's findings when they also reported loss of sensitivity to diuron in isolated PS II subparticles treated with low concentrations of trypsin. A decrease of 80-90% in diuron activity was reported after a 10 minute exposure of the PS II subparticles to trypsin. Gel electrophoresis of the particles revealed the loss of bands of approximately 27 and 32 kd. From this and other work (31, 35, 40) the concept of a proteinaceous binding site for the urea type herbicides has been firmly established.

Mechanisms of Inhibitory Activity. Identifying the nature of herbicide binding and the specific mechanisms of herbicide induced disruption of electron flow is now the main thrust of many investigations.

Moreland, in 1980 (30), noted the restoration of electron flow after washing of herbicide treated chloroplasts. This suggests, as reported previously (42, 43), that herbicide binding to the Q-B protein is not strong, i.e. not of a covalent nature. Moreland suggests that actual herbicide binding is between a complete charge transfer complex and a simple dipole interaction. This interaction may be reinforced by hydrogen bonds and is influenced by the nature of the lipophilic side chain of the herbicide molecule.

Shipman, in 1981 (38), published an excellent review article on the nature of herbicide binding, factors affecting that binding, and possible mechanisms of inhibitory activity. He suggests that herbicide binding to the Q-B protein shield is a dipole-dipole interaction between protein and herbicide molecules. He points out that all diuron type

herbicides (herbicides which bind to this same general area) possess dipole moments. The diuron type herbicides shift the $Q^{-}B \rightleftharpoons QB^{-}$ ratio to the left by interfering with the allosteric properties of the Q-B protein, thus inhibiting electron flow between the photosystems. Herbicide interactions with the Q-B protein may affect electron flow in one or more of the following ways: 1) by reducing the anion stabilizing properties of the Q-B protein (B has been shown to be stable in the semiquinone state for several seconds while acting as a gating mechanism for electron flow between photosystems (3, 46). Such stability is very unusual for a quinone in the lipid phase of the chloroplast membrane, thus it is thought that the Q-B protein may be important in a stabilizing role (32)), 2) by inhibiting important conformational changes or protonation of the Q-B protein in response to the reduction of B (which may relate to the stabilizing properties mentioned above), or 3) by displacing B from its binding site within the chloroplast membrane and the electron transport chain.

In the nature of the side chain, Shipman (38) states that it not only affects 1) access to the binding site (by influencing lipophilicity) and 2) proper positioning for binding, but also that the magnitude of the dipole as influenced by the side chain chemistry is of great importance to effective herbicide binding to the active site.

Papers by Van Rensen (45) and Arntzen et al. (1) also present excellent reviews of the theories and present knowledge of the mode of inhibitory action of the diuron type herbicides.

One final point has been raised in the investigation of diuron

type herbicide induced inhibition of photosynthesis. In a paper by Van Rensen and Vermass (44), the possibility of a key role of bicarbonate and/or CO_2 in photosynthetic electron transport is suggested. They feel that bicarbonate or CO_2 is needed by the Q-B protein for some yet unknown reason. Their work suggests that diuron type herbicides may interfere with the interactions of bicarbonate or CO_2 with the Q-B protein, affecting its function in regulating electron flow.

Overall it is evident that the mode of action of the ureas has not been fully elucidated. Several important questions remain. How does the Q-B protein regulate electron flow? Does herbicide binding result in some type of conformational or electrochemical alteration of this protein that prevents it from stabilizing a reduced quinone B? Is herbicide activity more related to a physical displacement of B from the Q-B protein by the herbicide molecule, or does the herbicide induce a loss of bicarbonate binding by the Q-B protein which prevents electron flow from occurring?

The series of experiments reported in this paper are not meant to answer the above questions. Rather the objectives of these experiments is to investigate the mode of action of one member of a new class of substituted urea herbicides, those containing an isoxazolyl ring substituent on the urea backbone. The mode of action of isouron, once determined, will aid in a further understanding of the nature of herbicide binding to the diuron site.

Isouron. Isouron was first synthesized by Shionogi and Company Ltd. in Japan (49). It is being marketed in the United States by Eli

Lilly and Company. Isouron is available as 50 WP, 75 WP, 1 G and 3 G formulations. It is also available in a premixed combination with tebuthiuron (N-(5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl)-N,N'-dimethylurea), oryzalin (3,5-dinitro-N^4,N^4-dipropylsulfanilamide) or atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine). Isouron is a broad spectrum, persistent herbicide, with a half-life in soil of 4-6 months. Significant weed control for a period of two months after application has been reported (49). Best control is achieved with preemergence or early postemergence application.

Isouron is recommended for use in noncropland areas at 1.12 to 4.48 kg a.i./ha for control of many annual grasses and broadleaf weeds and some perennial weeds. Isouron is also recommended for research in fallow lands. Applications in late summer or fall are suggested for annual grass and broadleaf control on fallowed land between crops of wheat (Triticum aestivum L.), corn, barley (Hordeum vulgare L.), grain sorghum, sunflower (Helianthus annuus L.) and rapeseed (Brassica napus L.). Suggested rates are 0.42-0.84 kg a.i./ha. Combinations with other herbicides are also recommended for research (22).

Herbicide Antidotes. The concept of a chemical herbicide antidote was first introduced in a 1962 publication by Otto Hoffman (15), although as early as 1947 he had observed antagonistic interactions between the herbicide 2,4-D (2,4-dichlorophenoxy acetic acid) and a chemical analog with very low phytotoxic activity, 2,4,6-T (2,4,6-trichlorophenoxy acetic acid), on tomato (Lycopersicon esculentum Mill.). Hoffman also experimented with the antidoting effects of 2,4-D on wheat from the effects of barban (4-chloro-2-butynyl N-(3-chlorophenyl)carbamate). In his 1962 publication, Hoffman proposed that antidotes could be beneficial to weed control programs in four ways: 1) by permitting the use of higher rates, which would normally cause crop injury, to allow for better weed control, 2) by preventing crop damage under adverse conditions where damage would otherwise be liable to occur, 3) by allowing the use of nonselective (or injurious) herbicides for selective weeding of susceptible crops, and 4) by providing protection to valuable plants that have been accidentally affected by a herbicide. To date, three of the four expectations have been realized, although these are limited to a few crops and a few herbicides. These gains justify continued work with herbicide antidotes, especially as the cost of developing new herbicides increases.

In discussing herbicide antidotes, the use of activated charcoal as a crop protectant should not be overlooked. In fact, activated charcoal could be considered as the first herbicide antidote. Its activity results from a physical adsorption of herbicides, thus preventing plant uptake (10, 18). For this reason, charcoal is perhaps not a true

chemical antidote, i.e. its protective properties do not result from chemical interactions with the herbicide within the plant. Activated charcoal is important though, in that this crop protectant is the only compound that has given susceptible crops adequate protection from the photosynthesis inhibitor type herbicides (1, 17, 21). The prohibitive cost of implementing a program of crop protection involving the use of activated charcoal has precluded its use in most field crops, although in situations involving high cash value crops such as strawberries (Fragaria x ananassa) (18), activated charcoal has shown some promise.

At present there are five commercially available chemical herbicide antidotes: NA, R-25788, CGA-43089, CGA-92194 (see Figure I-2), MON-4606. This paper reports work done involving the first four antidotes cited above. It is interesting and should be noted in the following discussion that only Graminaceous crops have been afforded significant protection by these antidotes from basically two classes of herbicides, the thiocarbamates (and to some extent the phenylcarbamates) and the acetanilides.

The first commercially available antidote was NA, released in 1971, as a protectant of corn against the herbicide EPTC (S-ethyl dipropylthiocarbamate). The protective action of NA does, however, extend to several other crops and several related herbicide families (3, 7, 16, 22, 29). Unfortunately, this protection is not economically feasible to register it for use in these crops. Crops protected by NA include corn, rice, sorghum, wheat, barley and oats (Avena sativa L.). Herbicide families antidoted significantly by NA include chloroacetanilides,

thiocarbamates and phenylcarbamates. Marginal results have been reported with amides and several miscellaneous groups of herbicides (14). NA is normally applied in the field as a seed dressing because of its relatively nonselective nature as a protectant, i.e. if weed seeds are treated, they too may be protected from herbicide injury.

The second herbicide antidote developed was R-25788, and its registered use is in corn to provide protection from the thiocarbamate herbicides EPTC and butylate (S-ethyl diisobutylthiocarbamate). The effectiveness of this antidote on corn is well documented (4, 5, 6, 24, 28). Protection to corn by R-25788 does extend to several herbicide families other than the thiocarbamates, specifically the acetanilides and phenylcarbamates (5, 6). R-25788 also provides partial protection to several crops other than corn, although this protection is not considered to be of economic value at the present time. Crops included in this group are sorghum (29) and oats (7). For a more complete listing of the range of R-25788 activity, one should refer to a review article by Hatzios (14) on herbicide antidotes. R-25788 is marketed as a premixed 12:1 solution (herbicide:antidote) and is applied in the field with the herbicide as a preplant incorporated treatment.

CGA-43089, the third antidote to be marketed, is registered for use only in grain sorghum for protection from metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide) injury, although significant protection has been reported for many of the other acetanilide herbicides (2, 9, 11, 13, 23, 25, 27, 30). This product is applied in the field as a seed treatment at approximately 1.25 g /kg seed.

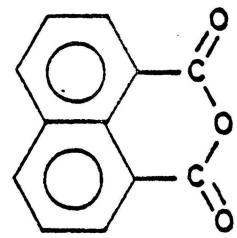
CGA-92194, a chemical analog of CGA-43089, is also used to protect sorghum from metolachlor injury. This product, however, has also provided significant protection from metolachlor to sweet sorghum, johnsongrass (Sorghum halepense L.) and sudangrass (Sorghum sudanese (Piper) Staph). CGA-92194 is also applied as a seed dressing, at rates of 1.5-2.0 g /kg seed.

MON-4606, which is not yet registered for commercial use as of August 1983, has been shown to be effective in sorghum providing protection from the chloroacetanilides alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide), acetochlor (2-chloro-N(ethoxymethyl)-6'-ethyl-o-acetotoluidide) and metolachlor.

The mode of action of the above mentioned herbicide antidotes is still basically unknown. Much work is being done in this area, but at present there are no definite theories as to their mode of action. One problem encountered in these types of investigations arises from the fact that the mode of action of the herbicides involved are not fully understood (32), which complicates investigations involving their corresponding antidotes. A discussion of the possible modes of action of these antidotes is outside the realm of this paper, however there is a large volume of published work dealing in this area (8, 12, 14, 16, 19, 20, 22, 26, 31).

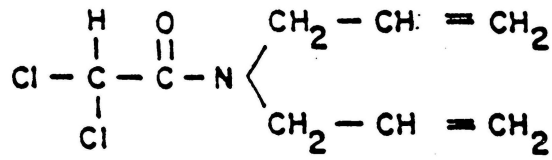
At present there are no antidotes available which are capable of significantly protecting crop plants from injury induced by photosynthetic inhibitor type herbicides, which comprise approximately 50% of the commercially used herbicides (1,29). Also there are no antidotes

available which can protect plants against the broad spectrum herbicides such as glyphosate or paraquat. If antidotes effective against one or both of the above mentioned herbicidal groups can be found, a major advance in the area of herbicide antidotes will be realized.



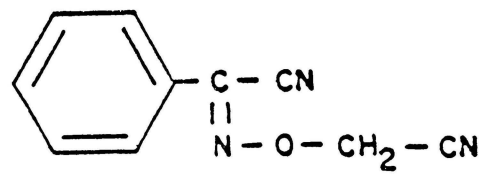
NA

1,8-naphthalic anhydride



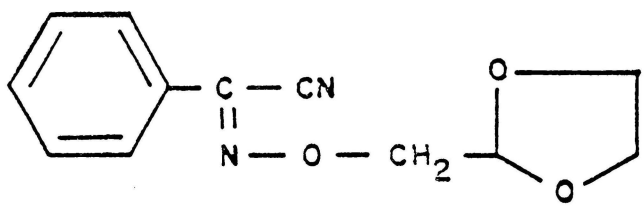
R-25788

N,N-diallyl-2,2-dichloroacetamide



CGA-43089

α-[(cyanomethoxy)imino]benzeneacetonitrile



CGA-92194

α-[(1,3-dioxolan-2-yl-methoxy)imino]benzeneacetonitrile

Figure I-2. Chemical structures of four comercial herbicide antidotes.

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II. EFFECT OF ISOURON AND TWO OF ITS PLANT METABOLITES ON FOUR METABOLIC PROCESSES IN ENZYMATICALLY ISOLATED SOYBEAN LEAF CELLS

Abstract. Laboratory studies were conducted to determine the effect of isouron and two of its plant metabolites on four metabolic processes, photosynthesis, protein synthesis, lipid synthesis, and ribonucleic acid (RNA) synthesis in enzymatically isolated soybean (Glycine max (L.) Merr.) leaf cells. Cell suspensions were incubated with radiolabeled precursors and various concentrations of the herbicide isouron and its monomethylurea and urea derivatives. Effects of these compounds on the four synthetic processes were determined by counting appropriately processed samples, taken at 30, 60 and 120 min intervals. The parent compound, isouron, was a potent inhibitor of all metabolic processes, as was its monomethylurea derivative. The urea derivative was least toxic to soybean cell metabolism, and in fact exhibited stimulation of three of the processes examined.

Isouron and the monomethylurea derivative effected the synthetic processes in the following order: Photosynthesis >lipid synthesis = RNA synthesis >protein synthesis. Over the intervals observed, time had little to do with the magnitude of inhibition of photosynthesis, lipid and RNA synthesis. Maximal inhibition occurred within 30 min for isouron and metabolite 1. Protein synthesis inhibition increased in magnitude from 30 to 60 to 120 min. Metabolite 2 (the urea derivative) exhibited significant inhibition of photosynthesis and RNA synthesis only, and the magnitude of inhibition was lower than that caused

by isouron or metabolite 1. There was little or no time dependence of the magnitude of inhibition with metabolite 2.

Laboratory studies were also performed to determine an I_{50} value for isouron. The data collected indicate the I_{50} concentration for isouron in isolated soybean leaf cells to be 0.51 uM. Further laboratory studies were carried out to determine the effect of the antioxidant EDU (N-(2-(2-oxo-1-imidazolidinyl)ethyl)-N'-phenylurea), and the singlet oxygen quencher, DABCO (1,4-diazobicylo-(2,2,2)-octane), on the toxicity of isouron over a 20 h test period. Also, a 24 h study was performed to observe the effect of EDU and DABCO on the chlorophyll content of isouron treated cells. No significant effect of these antioxidants was observed.

INTRODUCTION

Isouron is a member of the urea chemical family of herbicides, a group that is important in both field weed control programs and laboratory research. Most herbicides in this group have been shown to inhibit photosynthetic electron flow; only siduron (1-(2-methylcyclohexyl)-3-phenylurea) and chlorsulfuron (2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl)-benzenesulfonamide) do not inhibit photosynthesis (8). The ubiquitous nature of this mode of action within the family of ureas is believed to originate from the presence of the lactam moiety (14). Isouron is the only urea herbicide to have an isoxazolyl ring with an isobutyl group as a substitution to the urea molecule. It is known that different substituents at the R groups greatly affect

herbicidal activity of compounds containing the lactam moiety (5, 6, 13, 14), as reflected in the differences in I_{50} values recorded for the urea herbicides (3).

Research has shown that metabolism of the dimethyl ureas often results in altered phytotoxicity. The primary metabolic pathway of these compounds involves N-demethylation (10), and such a metabolic process has been shown to occur with isouron (11). Metabolism results in formation of a monomethyl urea derivative and often continues with the formation of a urea derivative.

The purpose of this research was to determine the mode of action of isouron by recording its effects on four synthetic processes in isolated soybean leaf cells. The relative phytotoxicity of the monomethylurea and urea derivatives of isouron was also investigated.

If photosynthetic inhibition was found as the mode of action, further investigations into the possible production of free radicals resulting from isouron treatments were to be conducted. Production of free radicals and resulting chlorophyll breakdown has been implicated as a possible mechanism of action of some urea herbicides (15, 16, 18).

METHODS AND MATERIALS

Plant Material. Soybean seeds were sown in plastic pots containing approximately 473 ml of soil mixture. The soil mix was a 2:2:1 blend of Weblite potting medium, vermiculite, and sphagnum peat moss and also contained a 4-9-4 commercial fertilizer, a controlled release 14-14-14 fertilizer and powdered limestone. Plants were thinned to one plant/pot

upon germination. Plants were allowed to grow for 5-10 days under greenhouse conditions and then placed into a growth chamber. The plants were provided with a light intensity of 28 klux during several days of 16 h photoperiod. After acclimation to growth chamber conditions, the 1 to 2 week old soybean plants were placed under a 6 h photoperiod for approximately five days prior to experimentation. A short day treatment has been reported to increase photosynthetic carbon fixation of isolated soybean leaf cells due to depletion of starch reserves (17). Growth chamber temperatures were the same for both photoperiods, 30° C day and 20° C night.

Cell Isolation. All procedures were carried out at room temperature. The method of isolation used was similar to that described by Jensen et al. (12) as modified by Ashton et al. (2). Healthy, 1 to 2 week old leaves were cut from the soybean plants. The detached leaves were rinsed with distilled water, blotted dry, deveined (major veins removed) and cut into strips approximately 1 mm X 1 cm with a razor blade. Two to three grams of tissue were then vacuum infiltrated with 30 ml of infiltration medium in a vacuum flask, for enough time to allow sufficient removal of air from the tissue and entry of the infiltration medium into the leaf pieces. The infiltration medium for all preparations contained 20 mM MES, pH 5.8, 2% Macerase (Calbiochem. Lajolla, California) and 0.3% potassium dextran sulfate (Calbiochem.). After infiltration was complete, the leaf tissue was filtered through a 242 uM nylon net, the filtrate discarded, and the leaf tissue remaining was transferred to a beaker to which 30 ml of maceration medium

was added. The maceration medium contained 20 mM MES, pH 5.8, 2% macerase, 0.3% potassium dextran sulfate, and 0.3 M sorbitol. The tissue was stirred in the beaker for 10 min with a magnetic stir bar. The resulting suspension was then filtered through the 242 uM nylon net and the filtrate discarded. The remaining tissue was transferred to another beaker and 30 ml of fresh maceration medium was added. This suspension was left to stir for approximately 1 h. The cells released during this 60 min treatment were collected by again filtering the suspension through the nylon net. The filtrate was equally distributed in three 15 ml centrifuge tubes and spun at 60 g for 3 min. The supernatant was removed by suction and the tube volumes were brought back to approximately 10 ml by adding a wash medium. The mixtures were stirred to resuspend the cells and then centrifuged for 3 min at 60 g. This washing procedure was repeated three times. The wash medium contained 0.2 M sorbitol, 5 mM KNC_3, 2 mM $Mg(NO_3)_2$ and 1 mM $CaCl_2$, and was buffered with 50 mM MES at pH 5.8 for protein, RNA, and lipid synthesis studies, and with 50 mM of HEPES at pH 7.8 for photosynthesis. After the final washing, the pellets were resuspended in wash medium to a desired volume and transferred to a beaker with agitation provided by a magnetic stirrer.

For chlorophyll determination, three 1-ml samples of the final cell suspension were placed into centrifuge tubes with 3 ml of 80% acetone in each. These were centrifuged at 60 g for 2 min and the supernatant fluid was then assayed spectrophotometrically for chlorophyll content according to the method of Arnon (1). The chlorophyll

content of cell preparations in this study was between 17 and 60 ug chl/ml assay medium.

Time Course and Concentration Studies With Isouron and its Two Plant Metabolites. 2 ml of the cell preparation, 0.1 ml of the correct radioactive substrate containing 1 uCi of radioactivity and 0.05 ml of herbicide solution were placed in a 25 ml erlenmyer flask in a similar fashion for all metabolic studies. Analytical grade (100%) isouron and its monomethylurea and urea derivatives were solubilized in methanol and brought to appropriate molar concentrations, resulting in a final concentration of methanol of less than 1%. Herbicide concentrations of 0.1, 1.0, 10.0 and 100.0 uM were used in all assays. The radioactive substrates used in each study were $NaH^{14}CO_3$ (sp. act. 44.4 mCi/mmol) for photosynthesis, L-(U-^{14}C) Leucine (sp. act. 290 mCi/mmol) for protein synthesis, (2-^{14}C) uracil (sp. act. 55 mCi/mmol) for RNA synthesis and (1,2^{14}C) acetic acid (sp. act. 56.2 mCi/mmol) for lipid synthesis. The erlenmyer flasks containing the herbicide cell suspension, and radioactive precursors were stoppered and placed in a slowly agitating shaker water bath at approximately 25° C and were exposed to 5.2 klux light provided by incandescent and fluorescent lights. Samples were taken from the flasks at 30, 60 and 120 min intervals. The samples taken were treated as described by the following procedures.

Photosynthesis. Photosynthesis was assayed according to the method of Jensen et al. (12) as modified by Ashton et al. (2). 100 ul samples were dripped onto 2.3 cm Whatmann 3 MM filter disks and allowed to dry

for approximately 30 min. After drying, the disks were acidified with 90% formic acid and allowed to dry. Radioactivity was determined by radioassay of the disks by liquid scintillation spectrometry. Photosynthetic rate was calculated as uM CO_2/mg chl/h.

Protein and RNA Synthesis. Assaying for these experiments involved the use of methods of Franki et al. (4) as modified by Ashton et al. (2). 500 ul samples were collected in 1.9 ml of ice cold 12% TCA (trichloroacetic acid) containing 50 mM leucine or 30 mM uracil for protein and RNA synthesis respectively. The samples were then left at 4° C overnight. The protein and RNA precipitates were collected by filtering through 2.1 cm glass fiber filter disks. The samples were washed three times with ice cold 10% TCA, three times with 80% ethanol, once with acetone and twice with diethylether. The disks were then placed in scintillation vials, dried for 30 min in an oven and radioactivity was measured by liquid scintillation spectrometry. The radioactivity was calculated as dpm (disintegrations per minute) per 100 ug of chlorophyll.

Lipid Synthesis. Lipid synthesis was assayed according to the method of Ashton et al. (2). 500 ul samples were collected in 2 ml of 0.35 M H_2SO_4 and 0.05 M CH_3COOH solution in conical centrifuge tubes. After sitting at least 15 min, the samples were centrifuged at 160 g for 10 min at room temperature. The supernatant was removed by suction and 4 ml of $CHCl_3$:CH_3OH (2:1) solution was added and mixed. Tubes were stoppered and left to sit overnight at room temperature. 2 ml of distilled H_2O was added to the tubes and they were

centrifuged at 160 g for 5 min at room temperature. The top layer of the supernatant was removed by suction. This procedure was repeated three times. After the third centrifugation, the upper aqueous layer was removed and the bottom lipid layer was passed through glass fiber filter disks into vials and the disks were washed three times with $CHCl_3$:CH_3OH (2:1). The filtered solution was allowed to dry under air and the radioactivity of the sample determined by liquid scintillation spectrometry. Radioactivity was calculated as dpm per 100 ug chlorophyll.

In all cases, 10 ml of aqueous counting scintillant (ACS, Amersham) were added to the samples in the vials and radioassay was done with a Beckman LS-250 series liquid scintillation spectrometer having a counting efficiency of 85% or higher. The results are listed in Tables 1-4. A percent inhibition value was calculated for each herbicide concentration using the control values as 0% inhibition. Data were subjected to analysis of variance and mean separation performed using Duncan's Multiple Range Test at the 5% level.

Free Radical Studies. From the data collected, it was evident that inhibition of photosynthesis was the primary mode of action of isouron; therefore, two additional studies were performed. The first was done to detect the effect of the antioxidant, EDU, and the singlet oxygen quencher, DABCO, on the photosynthetic inhibition by isouron as measured by uM CO_2/mg chl/h. In the second experiment the effect of these chemicals on the chlorophyll content of isolated soybean cells after a 24 h incubation was examined.

Antioxidant Effects on Isouron Toxicity. In this experiment, run over a 20 h time period, the effects of DABCO and EDU on photosynthetic inhibition caused by isouron were examined. Cells were isolated from soybeans as described earlier in this section, and sampling for photosynthesis effects was done in a similar fashion. Isouron was the only compound tested in this experiment; no metabolites were used. DABCO and EDU were added to the reaction mixtures at two different concentrations, 200 mM and 400 mM. Samples were taken at 2, 4, 8 and 20 h. The results are listed in Table 5. The data were subjected to analysis of variance and mean separation via Duncan's Multiple Range Test at the 5% level.

Antioxidant Effects on Chlorophyll Degradation. In this experiment the influence of DABCO and EDU on isouron treated soybean leaf cell chlorophyll content following a 24 h incubation period was examined. Chlorophyll content of the samples was determined after the 24 h incubation period by the method of Arnon (1) through procedures previously discussed. The data, given in ug chl/2 ml of cells, are presented in Table 6 and were subjected to analysis of variance and the Duncan's Multiple Range Test at the 5% level.

I_{50} Determination. Soybean leaf cells were isolated as previously described. Several herbicide concentrations were evaluated for their inhibitory effect. The concentrations used were 0.0, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 5.0 and 10.0 uM. Suspensions of isolated cells were incubated with $NaH^{14}CO_3$ and the various herbicide concentrations for 60 min. After this time, samples were taken and analyzed as

previously described. A percent of control value was calculated for each herbicide concentration. To determine the I_{50} for isouron, percent of control values were plotted versus log of herbicide concentration. The values in the graph represent average values determined from three replications of this experiment. From this graph, an I_{50} was calculated. (See Figure II-1.)

RESULTS AND DISCUSSION

Metabolic Studies. The effects of isouron, its monomethylurea (metabolite 1) and urea derivative (metabolite 2) on four metabolic processes of soybean leaf cells were examined. The data are listed in Tables 1-4. The parent compound and metabolite 1 exhibited similar effects in all four experimental assays. Metabolite 2 was found to be markedly less toxic to the metabolic processes of isolated soybean cells than isouron or metabolite 1. Photosynthesis appears to be most sensitive to all compounds tested, followed closely by lipid synthesis, then ribonucleic acid (RNA) synthesis, with protein synthesis inhibition being relatively minor after treatment with the three compounds.

Photosynthesis was inhibited 90 and 86% by the 100 uM concentration of isouron and metabolite 1 after a 30 min incubation. Metabolite 1 exhibited a greater toxic effect at the lower concentration, 10 uM, than isouron during the same time period. The inhibition increased to 96 and 95% for isouron and metabolite 1 respectively after 120 min. This was not a significant increase in inhibition over time. Thus it appears that the binding of isouron and its first metabolite to

Table 1. The effect of Isouron and two of its plant metabolites on photosynthesis of isolated soybean cells[a].

Incubation time (min)	Herbicide concentration (μM)	Isouron $^{14}CO_2$-fixation (μM CO_2/mg Chl/hr)	Isouron Inhibition (%)	Metabolite 1 $^{14}CO_2$-fixation (μM CO_2/mg Chl/hr)	Metabolite 1 Inhibition (%)	Metabolite 2 $^{14}CO_2$-fixation (μM CO_2/mg Chl/hr)	Metabolite 2 Inhibition (%)
30	0	13.19 ef	0	17.37 d	0	16.73 e	0
	0.1	11.73 efg	11	16.04 d	8	14.96 e	11
	1	8.63 efg	35	6.04 ef	65	15.71 e	6
	10	3.05 fg	77	2.64 f	85	16.62 e	1
	100	1.26 g	90	2.43 f	86	8.38 f	50
60	0	28.95 c	0	32.28 b	0	30.86 c	0
	0.1	24.15 cd	17	26.88 c	17	27.00 cd	13
	1	15.78 de	45	9.58 e	70	29.20 c	5
	10	4.49 fg	85	2.97 f	91	28.64 c	7
	100	1.77 g	94	2.86 f	91	13.58 ef	56
120	0	57.66 a	0	59.82 a	0	58.31 a	0
	0.1	44.93 b	22	55.29 a	8	51.53 b	12
	1	29.84 c	48	14.73 d	74	53.21 ab	9
	10	7.60 efg	87	3.64 f	94	52.67 ab	10
	100	2.12 g	96	2.93 f	95	22.57 d	61

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 2. The effect of Isouron and two of its metabolites on protein synthesis of isolated soybean cells[a].

Incubation time (min)	Herbicide concentration (μM)	Isouron [^{14}C]Leucine incorporated (dpm/100 μg Chl)	Isouron Inhibition[b] (%)	Metabolite 1 [^{14}C]Leucine incorporation (dpm/100 μg Chl)	Metabolite 1 Inhibition (%)	Metabolite 2 [^{14}C]Leucine incorporated (dpm/100 μg/ Chl)	Metabolite 2 Inhibition (%)
30	0	5,873 h	0	7,538 gh	0	7,840 ef	0
	0.1	6,108 h	-4	7,773 gh	-3	7,587 f	3
	1	5,103 h	13	6,880 ghi	9	9,968 def	-27
	10	4,818 h	18	5,189 hi	31	10,493 def	-34
	100	4,308 h	27	4,438 i	41	9,593 def	-22
60	0	15,208 e	0	16,379 e	0	18,326 cd	0
	0.1	14,282 ef	6	19,066 e	-16	17,753 cde	3
	1	11,862 fg	22	13,584 f	17	24,028 c	-31
	10	10,330 g	32	11,336 f	31	20,667 c	-13
	100	9,603 g	37	8,678 g	47	17,881 cde	2
120	0	36,353 a	0	35,171 b	0	40,157 ab	0
	0.1	30,266 b	17	44,196 a	-26	38,152 ab	5
	1	26,781 c	26	29,779 c	15	48,009 a	-20
	10	23,027 d	37	23,915 d	32	41,081 ab	-2
	100	15,459 e	58	17,531 e	50	37,700 b	6

[a]Means within columns followed by similar letters are not significantly different at the 5% Duncan's Multiple Range Test.

[b]A minus (-) sign in front of a percentage value indicates stimulation instead of inhibition.

Table 3. The effect of Isouron and two of its plant metabolites on ribonucleic acid synthesis of isolated soybean cells[a].

Incubation time (min)	Herbicide concentration (μM)	Isouron [^{14}C]Uracil incorporated (dpm/100 μg Chl)	Isouron Inhibition[b] (%)	Metabolite 1 [^{14}C]Uracil incorporated (dpm/100 μg Chl)	Metabolite 1 Inhibition (%)	Metabolite 2 [^{14}C]Uracil incorporated (dpm/100 μg Chl)	Metabolite 2 Inhibition (%)
30	0	10,651 cd	0	7,946 cd	0	8,452 ef	0
	0.1	10,779 cd	-1	8,443 c	-6	8,708 ef	-3
	1	4,656 e	56	6,031 cde	24	8,874 ef	-5
	10	2,818 e	74	2,972 e	63	7,039 f	17
	100	2,707 e	75	2,844 e	64	4,520 f	47
60	0	16,270 b	0	14,913 b	0	16,118 cd	0
	0.1	17,986 b	-11	14,773 b	1	15,664 cde	3
	1	7,240 de	56	7,941 cd	47	15,323 cde	5
	10	3,018 e	81	3,209 e	78	10,811 def	33
	100	2,880 e	82	2,708 e	82	6,597 f	59
120	0	28,737 a	0	24,821 a	0	29,024 a	0
	0.1	32,296 a	-12	26,188 a	-6	30,042 a	-4
	1	13,726 bc	52	15,892 b	36	24,878 ab	14
	10	3,169 e	89	3,889 de	84	20,073 bc	31
	100	3,414 e	88	3,521 de	86	10,116 def	65

[a]Means within columns followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

[b]A minus (-) sign in front of a percentage value indicates stimulation instead of inhibition.

Table 4. The effect of Isouron and two of its plant metabolites on lipid synthesis of isolated soybean cells[a].

Incubation time (min)	Herbicide concentration (μM)	Isouron [^{14}C]Acetate incorporated (dpm/100 μg Chl)	Isouron Inhibition[b] (%)	Metabolite 1 [^{14}C]Acetate incorporated (dpm/100 μg Chl)	Metabolite 1 Inhibition (%)	Metabolite 2 [^{14}C]Acetate incorporated (dpm/100 μg Chl)	Metabolite 2 Inhibition (%)
30	0	118,207 e	0	110,395 ef	0	128,250 d	0
	0.1	130,432 e	-10	117,893 ef	-6	140,885 d	-10
	1	104,286 ef	12	130,324 ef	-18	127,551 d	1
	10	81,454 efg	31	88,159 fg	20	121,128 d	6
	100	15,379 g	87	20,713 h	81	123,269 d	4
60	0	244,549 d	0	222,773 d	0	218,814 c	0
	0.1	274,553 d	-12	214,453 d	4	265,524 c	-21
	1	240,930 d	1	259,438 d	-16	212,643 c	3
	10	145,938 e	40	153,778 e	31	217,298 c	1
	100	32,431 fg	87	40,295 gh	82	236,521 c	-8
120	0	440,819 b	0	469,962 bc	0	491,986 b	0
	0.1	545,348 a	-24	503,700 ab	-7	564,591 a	-15
	1	588,160 a	-33	538,992 a	-15	523,507 ab	-6
	10	352,427 c	20	426,518 c	9	482,732 b	2
	100	66,162 efg	85	86,337 fg	82	519,145 ab	-6

[a]Means within columns followed by similar letters are not significantly different at the Duncan's Multiple Range Test.

[b]A minus (-) sign in front of a percentage value indicates stimulation instead of inhibition.

the active site is complete after 30 min. This was also noted with the 10 uM concentration. In contrast to the potent inhibitory effect of isouron and metabolite 1, metabolite 2 appears to be about 50-60% as toxic to the soybean leaf cells' photosynthetic processes, registering a 50% inhibition at 30 min and climbing only to 61% after 120 min incubation. As in the case of isouron and metabolite 1, it appears that the binding of metabolite 2 is complete after 30 min.

Lipid synthesis appears slightly less sensitive to isouron and metabolite 1 than photosynthesis (Table 4). Metabolite 2 elicited stimulation rather than inhibition, although significant stimulation did occur with isouron and metabolite 1 at 120 min. The sharp and sustained inhibition of lipid synthesis registered by isouron and metabolite 1 at 100 uM may be a direct result of photosynthetic inhibition. Lipid synthesis is highly dependent on photosynthetic electron flow for both energy as ATP and reducing equivalents as NADH and NADPH. Similar results have been found in other experiments consisting of procedures similar to those carried out in this experiment (7, 8, 9). Compounds that inhibit photosynthesis often result in a marked inhibition of lipid synthesis as assayed by the procedures used in these experiments.

There may be another chemical interaction taking place between the compounds tested and lipid synthesis. Low inhibition values for herbicide concentrations below 100 uM and the repeated stimulation of lipid synthesis could indicate that isouron is acting directly on the lipid synthetic processes. This activity could be a direct stimulation

of lipid synthesis, or the effects of a toxin at low concentrations. It is a widely held principle that low concentrations of a toxin often result in stimulation of the process that the toxin affects. An examination of the data may help to substantiate this possibility. The stimulatory effects of isouron and its metabolites are best illustrated at the 120 min sampling period. While inhibition of photosynthesis is occurring as a result of isouron at the concentrations of 0.1 and 1.0 um, metabolite 1 at 1.0 um and metabolite 2 at 0.1 um, a significant stimulation of lipid synthesis is simultaneously recorded at these same concentrations and sampling period. Even taking into account other energy producing pathways such as glycolysis and the Kreb's Cycle, one would expect a great dependence of lipid synthesis on photosynthesis, especially when one takes into account the fact that initial biosynthesis of lipids occurs in the chloroplast. An exact biochemical explanation of this apparent stimulation of lipid synthesis is not readily available and any attempt at such would be conjecture. A more complete investigation of the interaction of these compounds and lipid synthesis could prove rewarding in this regard.

Ribonucleic acid synthesis appears to more closely follow the inhibition of photosynthesis by isouron and its two metabolites (Table 3). Although stimulation of RNA synthesis does occur at the lower herbicide concentrations, it is not significant. This notwithstanding, a fairly good correlation between the inhibition of RNA synthesis and photosynthesis does exist. Thus it would appear that the activity of isouron and its metabolites on RNA synthesis recorded in this experiment are a

direct result of the shortage of anabolic energy resulting from herbicidal effects on photosynthesis. As with photosynthesis, statistically complete inhibition of RNA synthesis was accomplished after 30 min with the absolute measure of synthesis, dpm/100 ug chl, increasing only slightly and not significantly at the 60 and 120 min samplings. Metabolite 2 showed no significant increase in total inhibition after 30 min , although the dpm/100 ug chl did increase more than two-fold from the 30 min to the 120 min sample.

The least sensitive process assayed was protein synthesis (Table 2), with a maximum of 58% inhibition recorded for isouron, 50% for metabolite 1 and 6% for metabolite 2. Metabolite 2 was far more stimulatory than inhibitory. It should be noted that the inhibition was not complete as it was in the photosynthesis assay. The dpm/100 ug chl recorded for the highest concentrations of isouron and metabolite 1 increased significantly with time, rising from 4,308 and 4,438 at 30 min to 9,603 and 8,678 at 60 min and to 15,459 and 17,531 at 120 min respectively. These data represent a linear increase over time, increasing by approximately a factor of 2 as incubation time increased two-fold. Such a relation would indicate that isouron and its monomethylurea derivative were not acting in a direct way to cause inhibition of the protein synthetic processes of the soybean cells. The inhibition recorded was probably an indirect result of the disruption of photosynthesis caused by isouron and metabolite 1, by reducing available anabolic energy. The results of metabolite 2 again are somewhat erratic. It appears this compound has little to no herbicidal

activity.

Calculation of I_{50} Value. The I_{50} concentration calculated for isouron is 0.51 uM (Figure II-1). This value is intermediate for the urea group of herbicides. Diuron and linuron have I_{50} values calculated to be approximately 0.19 uM while monuron and floumeturon possess values of 1.0 uM. Most I_{50} values are calculated using isolated chloroplasts, and the value calculated in this paper is for isolated cells. One might expect that an I_{50} for isouron in isolated chloroplasts would be somewhat lower than 0.51 uM; although in a Japanese study with isolated chloroplasts, a value of 0.93 uM was calculated for isouron (19). The methods used in the Japanese study are not readily available, however. In any case, it appears that isouron ranks as a relatively potent inhibitor of photosynthesis when compared to the other urea herbicides.

Antioxidant Effects. The experiments done with EDU and DABCO revealed that these antioxidants had no significant effect on toxicity of isouron or in prevention of chlorophyll breakdown during a 24 h period (Tables 5 and 6). This would indicate that the mode of action of isouron is not related to the oxidative breakdown of chlorophyll or membranes resulting from the production of free radicals.

Table 5. The effect of combined treatments of the herbicide isouron and the antioxidants DABCO and EDU on photosynthesis of isolated soybean leaf cells[a].

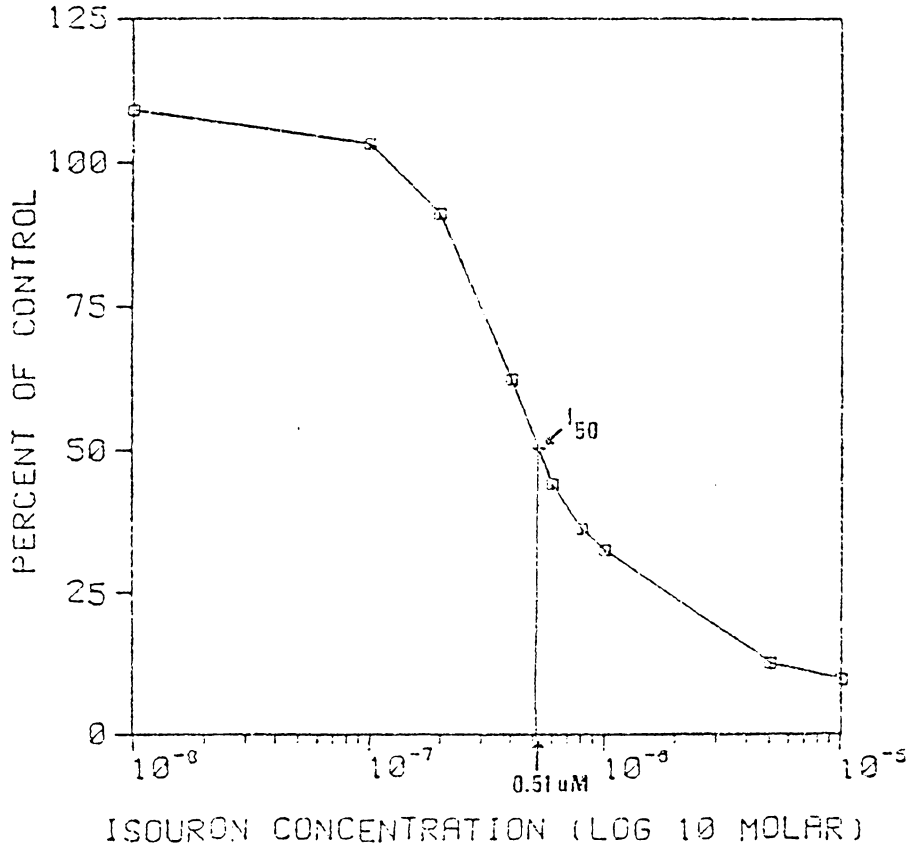
Incubation time (hr)	Isouron concentration (μM)	Antioxidant concentration (μM)				
			DABCO		EDU	
		0	10	100	10	100
		(uM CO_2/mg Chl)				
2	0	33.46 jkl	35.91 jk	35.08 jk	31.61 klm	32.38 jklm
	10	8.01 pqr	9.31 pqr	6.91 qr	8.58 pqr	9.85 pqr
	100	1.42 r	1.33 r	1.40 r	1.42 r	1.79 r
4	0	58.45 gh	68.64 f	62.86 fg	60.62 gh	54.59 h
	10	14.40 opq	14.91 opq	13.91 pq	15.21 opq	16.21 op
	100	1.54 r	1.52 r	1.76 r	1.72 r	2.13 r
8	0	98.97 d	111.10 c	101.90 d	99.39 d	87.62 e
	10	25.16 mn	26.80 lmn	22.00 no	26.30 lmn	25.21 mn
	100	1.83 r	1.90 r	2.16 r	2.08 r	2.36 r
20	0	127.44 b	139.05 a	140.77 a	126.78 b	115.76 c
	10	38.68 ijk	43.97 i	35.61 jk	40.45 ij	36.35 ijk
	100	2.58 r	2.97 r	2.92 r	2.86 r	2.85 r

[a]Means within columns and rows followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 6. The effect of combined treatments of the herbicide isouron and the antioxidants DABCO and EDU on the chlorophyll content of isolated soybean leaf cells following a 24 hr incubation[a].

	Antioxidant concentration (µM)				
Isouron		DABCO		EDU	
Concentration	0	200	400	200	400
(µM)	(µg chl/2 ml of cells)				
0	17.75 abc	15.90 b-e	16.60 a-e	18.35 ab	18.00 abc
1.0	18.00 abc	15.20 cde	15.45 b-e	19.65 a	17.30 a-d
10.0	15.70 de	14.05 e	13.75 e	16.65 a-e	14.60 de

[a]Means within columns and rows followed by similar letters are not significantly different at the 5% level by Duncan's Multiple Range Test.



II-1. Photosynthesis rate as percent of control versus log of Isouron concentration showing the intercept at 50 inhibition.

SUMMARY OF CELLULAR STUDIES

Isouron appears to inhibit photosynthesis as its primary mode of action. This finding is in agreement with preliminary studies done in Japan utilizing isolated chloroplasts (18). The I_{50} concentration determined in this study for isouron is 0.51 uM, as compared with 0.93 uM reported in the Japanese work. The I_{50} determined in this study, as compared to values of other urea derivatives (3), shows isouron as a fairly potent inhibitor of photosynthesis. In addition to the effects observed on photosynthesis, there may be an interaction of isouron and its metabolites with lipid synthesis, although this remains to be proved.

The two metabolites of isouron tested exhibit markedly different effects. Metabolite 1, the monomethylurea derivative, considered the first stable plant product in the sequential breakdown of isouron (10), is as toxic as the parent compound. Metabolite 2, the urea derivative, is believed to be derived from metabolite 1 by at least one metabolic step, i.e. it is a direct product of metabolism of metabolite 1. Metabolite 2 exhibits a greatly reduced toxic effect on the soybean cells' metabolic precesses, at times resulting in more stimulation that inhibition and appears to be a terminal metabolite of the herbicide isouron in plants.

Metabolism of the parent compound and metabolite 1 to the urea derivative by isolated soybean leaf cells appears to take longer than the 2 h. over which these cellular studies were performed. This

conclusion is drawn from the observation that inhibition of photosynthesis did not decline over the 2 h incubation period in both the isouron and metabolite 1 treatments. If metabolism to the urea derivative was occurring, a drop in observed inhibition would be expected, as the binding of urea herbicides to their active site in the photosynthetic electron chain protein is reversible. The minimal toxicity recorded for metabolite 2 could result from low affinity for the herbicide binding site.

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III. INTERACTIONS OF ISOURON WITH THE HERBICIDE ANTIDOTES NAPHTHALIC ANHYDRIDE, R-25788, CGA-43089 AND CGA-92194 ON TWO GREENHOUSE GROWN CORN AND WHEAT VARIETIES

Abstract. Greenhouse studies were carried out to investigate the effects of preemergence applications of isouron on the growth of 'XL67' and 'XL72AA' corn (Zea mays L.) and 'Tyler' and 'McNair 1003' wheat (Triticum aestivum L.). The effect of four herbicide antidotes on the toxicity of isouron to these corn and wheat varieties was also determined. The antidotes tested were naphthalic anhydride (1,8-naphthalic anhydride), R-25788 (N,N-diallyl-2,2-dichloroacetamide), CGA-43089 (α-((cyanomethoxy)imino)benzeneacetonitrile), and CGA-92194 (α-(1,3-dioxolan-2-yl-methoxy)imino)-benzeneacetonitrile). The herbicide isouron was applied as a preemergence spray at 0, 0.28, 0.56, 0.84 and 1.12 kg/ha. The antidotes naphthalic anhydride (NA), CGA-43089 and CGA-92194 were applied as seed treatments and R-25788 was applied as preplant incorporated spray.

Isouron proved to be very toxic to all varieties tested, eliciting severe stunting, chlorosis and necrosis. The degree and speed of symptom development was directly related to rate as evaluated by visual observation. The wheat varieties appeared to have little, if any, tolerance to isouron. The corn varieties exhibited more tolerance to isouron than wheat, although a high degree of toxicity to both crop species was evident. There was some antagonism of the toxic effects of isouron with the antidote treatments in corn and wheat, although this antagonism appeared only as slowing of symptom development and

did not appear to be of any practical agronomic importance. Some synergism between some antidotes and the low rates of the herbicide was observed in corn. Reduced germination was observed with the antidotes CGA-43089 and CGA-92194 in corn and wheat. The toxicity of CGA-92194 to both wheat varieties was great enough to make analysis of the results impossible. An overall assessment of the data reveals that the order of antidote effectiveness of the compounds tested is as follows: NA > CGA-43089 > R-25788 > CGA-92194. From the data, it would appear that further field trials involving isouron, the antidotes used, and the corn and wheat varieties tested, should not be performed due to the general toxicity displayed by isouron.

INTRODUCTION

As the cost of developing new selective herbicides continues to increase, the importance of herbicidal antidotes in modern weed control practices is becoming more evident. Isouron apparently lacks selectivity, hence its proposed use has been in fallowland and non-cropland weed control (3, 5). This herbicide is a potent inhibitor of photosynthesis and excellent herbicidal activity can be achieved at relatively low rates of field application. A compound such as isouron would therefore possess much greater effectiveness if some degree of selectivity could be induced, perhaps through the use of antidotes. At present, antidotes have shown little effectiveness in reducing phytotoxic properties of the photosynthesis inhibiting type herbicides (1, 2).

Antidotes have been used in the past to decrease the response of moderately susceptible plant species to several commercially important herbicides. Mostly these herbicides have been in the carbamate, thiocarbamate and acetanilide classes of compounds, although the commercially available antidotes have shown varying degrees of protection to herbicides in classes other than those mentioned above (1). An important step in antidote research would be accomplished if a compound was found to be effective in antidoting herbicides in the urea and/or triazine class. Isouron is unlike other urea herbicides due to the presence of an isoxyzolyl ring substituent. Due to the still imcomplete knowledge of the biochemical basis of antidotal activity, it is important that new herbicides, even those outside of the realm of those currently known to be successfully antidoted, be tested for interaction with available antidotal compounds. Whether successful or not, such trials will provide valuable information in the area of antidote mode of action. In this experiment, isouron was tested with four antidotes, NA, R-25788, CGA-43089 and CGA-92194 on two varieties of corn and wheat to determine what types of interactions would occur between these compounds.

METHODS AND MATERIALS

Greenhouse Studies. Two varieties of corn, a thiocarbamate-tolerant 'Dekalb XL72AA' and a thiocarbamate-susceptible 'XL67', and two varieties of wheat, 'Tyler' and 'McNair 1003', were used in this study. Plants were grown in 473 ml plastic pots with three plants per pot.

The growth medium was a 2:2:1 mixture of Weblite, vermiculite and peat-moss. This mixture contained a 4-9-4 agricultural fertilizer, a 14-14-14 slow release fertilizer and limestone. All greenhouse experiments were carried out during the summer of 1983. The herbicide isouron was formulated as a 50% wettable powder. The antidotes NA, CGA-43089 and CGA-92194 were technical grade compounds applied as seed dressings at the recommended rates of 0.5 and 1.0% w/w, 1.25 g compounds/kg seed and 1.5 and 2.0 g compound/kg seed respectively. The seed treatment procedure was performed as follows. Appropriate weights of the antidotes were weighed and added to an erlenmeyer flask containing the seeds to be coated. Ten ml of ethanol was added to the flask to solubilize the antidote and the flask was shaken until dry, resulting in an even application to all seeds. A rotating belt sprayer was used to make the R-25788 and isouron applications. The R-25788 was formulated as a 6.7 lb/gal emulsifiable concentrate. Rates of 0.6 and 1.2 kg/ha R-25788 were applied over the top of the 473 ml plastic pots filled to appropriate volume with potting medium. Immediately following application of the R-25788, the upper 2 to 3 cm of potting medium was removed and shaken vigorously in a plastic tray to incorporate the antidote. Untreated corn or wheat seed was then placed into the partially full pots and the antidote treated soil was placed back into the pots to cover the seeds. Corn and wheat seed treated with the respective antidotes were placed into pots and covered with untreated soil. Isouron treatments were then applied. Isouron was applied as a preemergence spray to these pots at 0.28, 0.56, 0.84 and 1.12 kg/ha.

Controls were run for herbicide and all antidote applications. All experiments were conducted three times with two replications in each experiment.

Analysis of Data. After three weeks growing time, plants were harvested. A razor was used to cut the plants off at the soil surface. At the time of harvest, plant height and fresh weight were recorded. Samples were placed in a forced air oven at 70° C for at least 48 h after which dry weights were taken. Analysis of dry weights was considered to be the most accurate variable to evaluate. Mean weight values were calculated for each treatment from the three duplicated experiments, as well as the standard error of each mean. F-values for each combination treatment were calculated for 2 by 2 comparison of that treatment with the control and the separate levels of isouron and antidote involved. Expected values for each treatment were determined by the use of the additive model (4).

RESULTS AND DISCUSSION

Corn Experiments. The results of greenhouse experiments performed on the corn varieties 'XL72AA' and 'XL67' are presented in Tables 1 and 2 respectively. Isouron exhibited a high degree of toxicity to both corn varieties, when no antidotes were applied, at rates greater than 0.28 kg/ha. This toxicity was almost complete at the highest rate of 1.12 kg/ha, resulting in severely stunted and often totally dead plants. There appeared to be no varietal differences in response to non-antidoted isouron applications. Naphthalic

Table 1. Shoot dry-weights of 'XL72AA' corn seedlings treated with preemergence applied isouron and the antidotes NA, R-25788, CGA-43089, and CGA-92194[a].

Antidote	Type of response	Isouron (kg/ha)				
		0	0.28	0.56	0.84	1.12
		(g)				
NA (%, w/w)						
0.0	Observed[b]	2.75 ± 0.31	2.72 ± 0.37	1.75 ± 0.34	1.02 ± 0.30	0.23 ± 0.05
0.5	Observed	1.83 ± 0.05	1.50 ± 0.20	1.35** ± 0.30	1.08** ± 0.21	0.58** ± 0.07
	Expected[c]		(1.80)	(0.83)	(0.10)	(-0.69)
1.0	Observed	1.80 ± 0.21	1.50 ± 0.17	1.50** ± 0.26	1.0/** ± 0.18	0.75** ± 0.21
	Expected		(1.77)	(0.80)	(0.07)	(-0.72)
R-25788 (kg/ha)						
0.0	Observed	2.75 ± 0.31	2.72 ± 0.37	1.75 ± 0.34	1.02 ± 0.30	0.23 ± 0.05
0.6	Observed	2.83 ± 0.21	3.33** ± 0.25	1.58 ± 0.29	0.72* ± 0.11	0.27 ± 0.08
			(2.80)	(1.83)	(1.10)	(0.31)
1.2	Observed	2.78 ± 0.27	2.72 ± 0.34	1.53 ± 0.20	0.58**± 0.15	0.22 ± 0.04
	Expected		(2.75)	(1.78)	(1.05)	(0.26)
CGA-43089, (g/kg seed)						
0.0	Observed	2.95 ± 0.25	2.85 ± 0.16	2.22 ± 0.19	1.27 ± 0.22	0.52 ± 0.09
1.25	Observed	1.70 ± 0.15	1.93* ± 0.13	1.50** ± 0.46	0.48** ± 0.18	0.22** ± 0.49
	Expected		(1.60)	(0.97)	(0.02)	(-0.73)
CGA-92194 (g/kg seed)						
0.0	Observed	2.95 ± 0.25	2.85 ± 0.16	2.22 ± 0.19	1.27 ± 0.22	0.52 ± 0.09
1.5	Observed	2.10 ± 0.25	2.22 ± 0.25	1.48 ± 0.16	0.82 ± 0.22	0.37 ± 0.03
	Expected		(2.00)	(1.37)	(0.42)	(-0.33)
2.0	Observed	2.38 ± 0.11	1.85 ± 0.32	1.32 ± 0.35	1.00 ± 0.17	0.78 ± 0.26
	Expected		(2.28)	(1.65)	(0.70)	(0.05)

[a]Data represent dry matter accumulation of corn seedlings during a 21-day period.

[b]Mean weight values are from six replications ± standard error of each mean. Asterisks indicate significant interactions at the 5% (*) or 1% (**) level of probability as determined by t-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of isouron and antidote involved.

[c]Expected values in parenthesis were calculated assuming no interactions (See Materials and Methods).

Table 2. Shoot dry-weights of 'X167' corn seedlings treated with preemergence applied isouron and the antidotes NA, R-25788, CGA-43089, and CGA-92194[a].

Antidote	Type of response	Isouron (kg/ha) 0.00	0.28	0.56	0.84	1.12
		(g)				
NA (1, w/w)						
0.0	Observed[b]	3.02 ± 0.13	3.22 ± 0.15	2.38 ± 0.23	1.32 ± 0.25	0.65 ± 0.14
0.5	Observed	2.35 ± 0.16	2.08** ± 0.28	1.62 ± 0.19	1.15** ± 0.13	1.07** ± 0.26
	Expected[c]		(2.55)	(1.71)	(0.65)	(-0.02)
1.0	Observed	2.17 ± 0.18	2.05* ± 0.22	1.85* ± 0.11	1.27** ± 0.15	0.85** ± 0.13
	Expected		(2.37)	(1.53)	(0.47)	(-0.20)
R-25788 (kg/ha)						
0.0	Observed	3.02 ± 0.13	3.22 ± 0.15	2.38 ± 0.23	1.32 ± 0.25	0.65 ± 0.14
0.6	Observed	3.67 ± 0.28	3.80 ± 0.18	3.07 ± 0.28	1.63 ± 0.52	0.87* ± 0.34
	Expected		(3.87)	(3.03)	(1.97)	(1.30)
1.2	Observed	3.13 ± 0.10	3.30 ± 0.23	2.37 ± 0.16	1.58 ± 0.35	0.85 ± 0.23
	Expected		(3.33)	(2.49)	(1.43)	(0.76)
CGA-43089 (g/kg seed)						
0.0	Observed	3.02 ± 0.13	3.32 ± 0.12	2.45 ± 0.31	0.97 ± 0.18	0.33 ± 0.10
1.25	Observed	2.10 ± 0.41	1.60** ± 0.80	0.98** ± 0.28	0.33 ± 0.06	0.32** ± 0.07
	Expected		(2.40)	(1.53)	(0.05)	(-0.59)
CGA-92194 (g/kg seed)						
0.0	Observed	3.02 ± 0.13	3.32 ± 0.12	2.45 ± 0.31	0.97 ± 0.18	0.33 ± 0.10
1.5	Observed	2.68 ± 0.22	2.15** ± 0.19	1.47** ± 0.22	0.65 ± 0.12	0.42** ± 0.13
	Expected		(2.98)	(2.11)	(0.63)	(-0.01)
2.0	Observed	2.86 ± 0.18	2.63 ± 0.23	1.83 ± 0.15	1.18 ± 0.33	0.65 ± 0.22
	Expected		(3.16)	(2.29)	(0.81)	(0.17)

[a]Data represent dry matter accumulation of corn seedlings during a 21-day period.

[b]Mean weight values are from six replications ± standard error of each mean. Asterisks indicate significant interactions at the 5% (*) or 1% (**) level of probability as determined by F-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of isouron and antidote involved.

[c]Expected values in parenthesis were calculated assuming no interactions (See Materials and Methods).

anhydride exhibited the most significant protection against isouron injury in both corn varieties. Despite two instances of synergistic interaction ('XL67' at the 0.28 kg rate of isouron), significant antagonism was noted at the higher rates of isouron in both varieties. The results indicate that NA afforded the corn statistically significant protection, however visual observation of this protection indicated that the effect of NA was only to slow symptom development. Perhaps in time the corn plants could outgrow the effects of isouron with the protection afforded by NA, however an early season delay in crop establishment resulting from this period needed to outgrow symptoms would surely impair the effectiveness of such a herbicide antidote program if one was implemented in the field. Still it is significant to note that NA did have an antagonistic effect on the toxicity of this photosynthesis inhibiting herbicide.

Among the other three antidotes tested, only CGA-43089 had any consistent effect in decreasing toxicity of isouron to these two corn varieties. Variety 'XL72AA' exhibited significant protection at all rates of isouron when treated with CGA-43089, while variety 'XL67' was adversely and synergistically affected by this antidote at two low rates of isouron (0.28 and 0.56 kg/ha) with a significant antidoting being observed at the 1.12 kg/ha isouron rate. As in the case of NA, the observed antagonism would appear to be of only academic interest owing to the possible economic problems of attempting to use this program for protecting corn from isouron in a field situation.

The antidotes which exhibited the least protection of corn from

isouron toxicity were R-25788 and CGA-92194. There were no instances where R-25788 antagonized herbicidal effects. Some synergism was observed with R-25788 and isouron. CGA-92194 protected corn in only one instance, at the 1.12 kg/ha isouron rate and 1.5 g/kg seed antidote rate. From this, it appears that neither of these two antidotes had any important antidotal effects on these corn varieties and isouron.

In conclusion, then, it appears the antidotal properties of the compounds tested on varieties of corn treated with isouron are, in order of decreasing effectiveness, NA, CGA-43089, CGA-92194 and R-25788. Of the two corn varieties tested, the thiocarbamate-tolerant variety 'XL72AA' appears to respond somewhat better to the antidotes that the thiocarbamate-susceptible variety, XL67.

Wheat Experiments. The results of the wheat experiments, involving the varieties 'Tyler' and 'McNair 1003', are presented in Tables 3 and 4 respectively. As in the corn experiments, the toxicity of isouron to these wheat varieties is quite evident. Non-antidoted herbicide applications resulted in progressively less dry weight accumulation during the three week growing period as the isouron rates increased. Symptom development was very similar to that in corn, however this development was accelerated and somewhat more acute in wheat. The more acute reaction of wheat to isouron could be related to seed size, with corn having more stored energy which would increase seedling vigor.

In terms of antagonism between the antidotes tested and isouron treatments, NA was by far the most potent in this respect. In fact,

Table 3. Shoot dry-weights of 'Tyler' wheat seedlings treated with preemergence applied isouron and the antidotes NA, R-25788, CGA-43089, and CGA-92194[a].

Antidote	Type of response	Isouron (kg/ha) 0.0	0.28	0.56	0.84	1.12
		(g)				
NA (%, w/w)						
0.0	Observed[b]	0.230 ± 0.028	0.142 ± 0.029	0.077 ± 0.023	0.043 ± 0.009	0.025 ± 0.002
0.5	Observed	0.050 ± 0.000	0.095** ± 0.026	0.040** ± 0.021	0.010** ± 0.000	0.007** ± 0.015
	Expected[c]		(-0.038)	(-0.103)	(-0.137)	(-0.155)
1.0	Observed	0.074 ± 0.014	0.077** ± 0.019	0.014** ± 0.002	0.014** ± 0.002	0.013** ± 0.003
	Expected		(-0.014)	(-0.079)	(-0.113)	(-0.131)
R-25788 (kg/ha)						
0.0	Observed	0.230 ± 0.028	0.142 ± 0.029	0.077 ± 0.023	0.043 ± 0.009	0.025 ± 0.002
0.6	Observed	0.255 ± 0.033	0.147 ± 0.027	0.115 ± 0.028	0.040 ± 0.014	0.018 ± 0.003
	Expected		(0.167)	(0.102)	(0.068)	(0.050)
1.2	Observed	0.235 ± 0.018	0.137 ± 0.026	0.080 ± 0.025	0.033 ± 0.010	0.013 ± 0.002
	Expected		(0.147)	(0.082)	(0.048)	(0.030)
CGA-43089 (g/kg seed)						
0.0	Observed	0.268 ± 0.030	0.202 ± 0.038	0.077 ± 0.018	0.023 ± 0.007	0.025 ± 0.003
1.25	Observed	0.140 ± 0.044	0.092 ± 0.008	0.028** ± 0.010	0.027** ± 0.013	0.015** ± 0.002
	Expected		(0.074)	(-0.051)	(-0.105)	(-0.103)

[a]Data represent dry matter accumulation of wheat seedlings during a 21-day period.

[b]Mean weight values are from six replications ± standard error of each mean. Astericks indicate significant interactions at the 5% (*) or 1% (**) level of probability as determined by F-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of isouron and antidote involved.

[c]Expected values in parentheses were calculated assuming no interactions (See Materials and Methods).

Table 4. Shoot dry weights of 'McNair 1003' wheat seedlings treated with preemergence applied isouron and the antidotes NA, R-25788, CGA-43089, and CGA-92194[a].

Antidote	Type of response	Isouron (kg/ha) 0.0	0.28	0.56	0.84	1.12
		(g)				
NA (%, w/w)						
0.0	Observed[b]	0.192 ± 0.009	0.120 ± 0.016	0.097 ± 0.026	0.037 ± 0.009	0.022 ± 0.004
0.5	Observed	0.100 ± 0.029	0.102** ± 0.017	0.040** ± 0.016	0.018** ± 0.003	0.015** ± 0.003
	Expected[c]		(0.028)	(0.005)	(-0.055)	(-0.070)
1.0	Observed	0.072 ± 0.023	0.040** ± 0.011	0.047** ± 0.016	0.022** ± 0.008	0.015** ± 0.003
	Expected		(0.000)	(-0.023)	(-0.083)	(-0.098)
R-25788 (kg/ha)						
0.0	Observed	0.192 ± 0.009	0.120 ± 0.016	0.097 ± 0.026	0.037 ± 0.009	0.022 ± 0.004
0.6	Observed	0.168 ± 0.019	0.123** ± 0.011	0.082 ± 0.013	0.063** ± 0.013	0.043** ± 0.014
	Expected		(0.096)	(0.073)	(0.013)	(-0.002)
1.2	Observed	0.175 ± 0.019	0.119 ± 0.021	0.058 ± 0.011	0.047** ± 0.009	0.023 ± 0.004
	Expected		(0.103)	(0.080)	(0.020)	(0.005)
CGA-43089 (g/kg seed)						
0.0	Observed	0.185 ± 0.038	0.078 ± 0.014	0.090 ± 0.020	0.037 ± 0.012	0.037 ± 0.009
1.25	Observed	0.143 ± 0.020	0.092** ± 0.024	0.050 ± 0.015	0.040** ± 0.010	0.035** ± 0.013
	Expected		(0.036)	(0.048)	(-0.005)	(-0.005)

[a]Data represent dry matter accumulation of wheat seedlings during a 21-day period.

[b]Mean weight values are from six replications ± standard error of each mean. Asterisks indicate significant interaction at the 5% (*) or 1% (**) level of probability as determined by F-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of isouron and antidote involved.

[c]Expected values in parentheses were calculated assuming no interactions (See Materials and Methods).

the protection afforded to both wheat varieties by NA was significant at the 0.01 level for all herbicide and antidote treatments. As in the corn experiment, however, this finding appears to be of only academic interest, as the injury to the wheat plants was well above levels which could be commercially acceptable.

Treatment of seeds with CGA-43089 also caused significant antagonism of isouron effects. Only two treatments were not antagonistic in variety 'Tyler' at 0.28 kg/ha isouron and in 'McNair 1003' at the 0.56 kg/ha herbicidal rate. To reiterate, it should be noted that the wheat plants did not appear healthy in any of the herbicide treatments. The antidote appeared to slow the development of symptoms, indicating some interference with herbicidal activity.

Soil applied treatments of R-25788 had no protective effect in the 'Tyler' trials, although four instances of significant antagonism were observed in the 'McNair 1003' trials. Perhaps a seed treatment with R-25788 may have improved its antidotal performance by ensuring a greater seed-antidote contact.

Summarizing the wheat experiments, NA and CGA-43089 appear to have statistically significant antidotal effectiveness against isouron induced toxicity to the wheat varieties tested. R-25788 exhibits limited protective properties in combination with isouron on these wheat varieties. CGA-92194 was too toxic to the wheat varieties used, severely limiting germination and making analysis of results impossible.

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PHYSIOLOGICAL STUDIES WITH THE

EXPERIMENTAL HERBICIDE ISOURON

by

William T. Henry

(ABSTRACT)

Laboratory studies were conducted to investigate the mode of action of a new herbicide, isouron (N-(5-(1,1-dimethylethyl)-3-isoxazolyl)-N,N-dimethylurea). These studies included two plant metabolites of isouron, a monomethylurea (N-(5-(1,1-dimethylethyl)-3-isoxazolyl)-N-methylurea) and a urea derivative (N-(5-(1,1-dimethylethyl)-3-isoxazolyl)-urea). The compounds were examined for their effects on four metabolic processes of enzymatically isolated soybean (Glycine max L.) leaf cells. The processes examined were photosynthesis, protein, ribonucleic acid (RNA) and lipid synthesis. Relative incorporation of radiolabeled precursors was the parameter measured to assess herbicidal effects. Results indicate that isouron and its monomethylurea derivative possess similar herbicidal properties. The urea derivative revealed substantially reduced herbicidal effectiveness. The primary mode of action of these compounds appears to be inhibition of photosynthesis.

Experiments to determine an I_{50} value for isouron and experiments to evaluate the influence of an antioxidant EDU (N-(2-(2-oxo-1-imidazolidinyl)ethyl)-N'-phenylurea) and a singlet oxygen quencher DABCO (1,4-diazobicyclo-(2,2,2)-octane) on the photosynthetic inhibition

and chlorophyll breakdown induced by isouron were also conducted. An I_{50} value of 0.51 uM was calculated for isouron. There was no significant influence of EDU or DABCO on isouron toxicity.

Greenhouse experiments were conducted to assess the interactions of isouron and four herbicide antidotes on two varieties of corn (Zea mays L.), Dekalb 'XL72AA' and 'XL67', and wheat (Triticum aestivum L.), 'Tyler' and 'McNair 1003'. Antidotes used were naphthalic anhydride (1,8 naphthalic anhydride), R-25788 (N,N-diallyl-2,2-dichloroacetamide), CGA-43089 (α-((cyanomethoxy)imino)benzene acetonitrile) and CGA-92194 (α-((1,3-dioxolan-2-yl-methoxy)imino)benzene acetonitrile. Statistically significant antagonistic interactions between herbicide and antidote were observed in both wheat and corn. Synergistic interactions were also recorded. The order of antidotal effectiveness on corn was determined to be: NA > CGA-43089 > CGA-92194 > R-25788 and in wheat: NA > CGA-43089 > R-25788 (CGA-92194 severely limited germination). Visual observation of both corn and wheat plants indicated that nonantidoted isouron was very toxic to these crops.