Comparative Studies on the Modes of Action of SC-0224 and Glyphosate

by

William Edward Cooley

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in
Plant Physiology / Weed Science

APPROVED:

Chester L. Foy, Chairman

John L. Hess

Laurence D. Moore

Maynard G. Hale

Kriton K. Hatzios

May 1985

Blacksburg, Virginia

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(ABSTRACT)

The biological actions of the herbicides SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) and glyphosate [N-(phosphonomethyl)glycine] (PMG) were compared. In each study trimethylsulfonium iodide (TMS-I) was included as a treatment because the trimethylsulfonium ion is a constituent of the SC-0224 molecular structure.

In inflated duckweed (Lemna gibba L.), both formulated and technical grade forms of SC-0224 were found to be much more phytotoxic to duckweed than either formulated or technical grade forms of glyphosate. The growth inhibition caused by glyphosate was partially prevented by different combinations of the aromatic amino acids phenylalanine, tyrosine, and tryptophan; whereas, the duckweed growth inhibition caused by SC-0224 could not be reduced by the same amino acid combinations. TMS-I and SC-0224 were found to be equally phytotoxic to duckweed. SC-0224 caused larger increases than glyphosate in the pool levels of amino acids; the increases caused by SC-0224 were similar, however, to those caused by trimethylsulfonium iodide. Expressed on a per gram fresh weight basis none of the chemical treatments caused significant changes in soluble protein or the incorporation of ^{14}C-leucine into soluble pro-

tein. On a per flask basis (allowing for decreased growth in treated flasks), both herbicides and TMS-I caused significant decreases in soluble protein and ^{14}C-leucine incorporation. SC-0224 and TMS-I caused larger decreases than glyphosate in both cases but the SC-0224 and TMS-I treatments were not significantly different. These data indicate that differences in the phytotoxicity of SC-0224 and glyphosate may be due to the action of the trimethylsulfonium ion of the SC-0224 structure.

The effects of these herbicides on the conversion of shikimate to anthranilate in a cell-free extract of Klebsiella pneumoniae ATCC 25306 were also compared. SC-0224 and glyphosate equally inhibited the production of anthranilate indicating that SC-0224 has action similar to glyphosate on the shikimate pathway.

The effects of these herbicides on photosynthetic electron transport (the Hill reaction) was determined using isolated thylakoids from Alaska pea (Pisum sativum L.). The action of SC-0224 was compared with the action of glyphosate, TMS-I and diuron [3-(3,4-dichorophenyl)-1,1-dimethylurea]. SC-0224, glyphosate and TMS-I did not inhibit the Hill reaction at concentrations up to 10 mM; whereas, diuron caused an almost total inhibition at 0.10 mM. The results of this study indicate that SC-0224 is not an inhibitor of photosynthetic electron transport.

These studies indicate that both constituents of the SC-0224 structure, TMS and PMG, are phytotoxic and may act independently.

ACKNOWLEDGEMENTS

I sincerely thank Dr. C. L. Foy, my major advisor, for the guidance, advice and financial support provided during the course of this research. For their various contributions of time, advice, and laboratory space, I thank my other committee members, Drs. J. L. Hess, K. K. Hatzios, M. G. Hale, and L. D. Moore. I extend my gratitude to Drs. G. H. Lacy, S. A. Tolin, and S. A. Alexander for making their laboratories available for portions of this research. I also thank Dr. E. S. Hagood for providing valuable training, advice, and assistance and for making his computer terminal available to me. The entire faculty, students, and staff of the department of Plant Pathology, Physiology and Weed Science have been helpful and have contributed in various ways to my program. For their invaluable moral support and companionship during difficult times, I express sincere gratitude to my good friends

and Finally, I express my deepest thanks to my parents,

and and my sister, for their unwavering support, encouragement, and sacrifices during the entire project.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

I. LITERATURE REVIEW AND OBJECTIVES

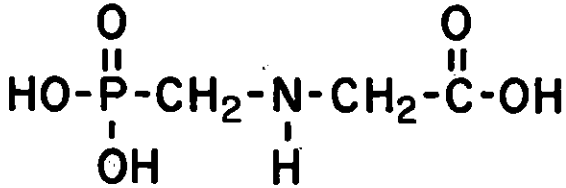
The action of a herbicide on a plant involves a number of physiological and morphological changes which may be triggered by the action of the chemical at some primary site of action. Identifying this primary site is difficult due to the complexity of the interactions of the many biochemical and physiological processes in plants. There is, however, increasing interest in determining the primary site of action of herbicides for a number of reasons. Increasing cost of herbicide development has caused the agricultural chemical industry to increase its emphasis on biorational approaches to herbicide design in place of traditional screening methods and knowledge of the biochemical action of herbicides is needed for these approaches. This type of information is also helpful in localizing genes which code for the site of action of herbicides. Altered genes may subsequently be used to confer herbicide resistance on crop species by use of recombinant DNA procedures (27). Mechanism of action data may also be useful to chemical companies in their efforts to gain registration for commercial use of a compound and to gain and/or defend patents. Site of action studies have also provided important information and research tools for scientists interested in more basic aspects of plant physiology and biochemistry.

SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) is an experimental postemergence, nonselective, translocatable herbicide which is structurally related to glyphosate [N-(phosphonomethyl)glycine (PMG). Glyphosate is commercially formulated as the isopropylamine salt

of PMG, whereas SC-0224 is its trimethylsulfonium salt (Figure 1). Trimethylsulfonium salts have previously been shown to have phytotoxic effects on a number of plant species and to exhibit selectivity in the magnitude of this phytotoxicity among species. Species demonstrating varying degrees of sensitivity included bermudagrass [Cynodon dactylon (L.) Pers.], carpetgrass [Axonopus compressus (Sw.) Beauv.], maize (Zea mays L.), cotton (Gossypium hirsutum L.), redroot pigweed (Amaranthus retroflexus L.), and common duckweed (Lemna minor L.) (59). Species tolerant to trimethylsulfonium salts included radish (Raphanus sativus L.), mustard (Brassica nigra L.), barley (Hordeum vulgaris L.), and soybean [Glycine max (L.) Merr.].

Previous laboratory, greenhouse, and field studies have shown SC-0224 to have metabolic and phytotoxic effects similar to those of glyposate although differences have occurred in some instances (7, 8, 12, 18, 19, 30, 35, 40, 67). One field study comparing the efficacy of SC-0224 and glyphosate for control of quackgrass [Agropyron repens (L.) Beauv.] demonstrated no difference between the two compounds (40). Field and greenhouse studies with hard red winter wheat (Triticum aestivum L. 'Centurk 78') also showed no difference in the phytotoxicity of the two compounds (12). Results from other field studies which compared the efficacy of the two herbicides for control of horseweed [Conyza canadensis (L.) Cronq.], common ragweed (Ambrosia artemisiifolia L.), rye (Secale cereale L.), fall panicum (Panicum dichotomiflorum Michx.), Pennsylvania smartweed (Polygonum pensylvanicum L.), common lambsquarters (Chenopodium album L.), large crabgrass [Digitaria sanguinalis (L.) Scop.], and redroot pigweed demonstrated that the activity of SC-0224 closely mir-

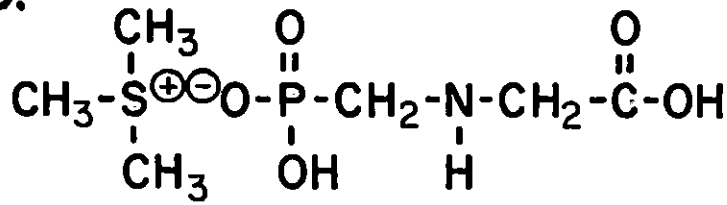
A.



Glyphosate

N-(phosphonomethyl)glycine

B.

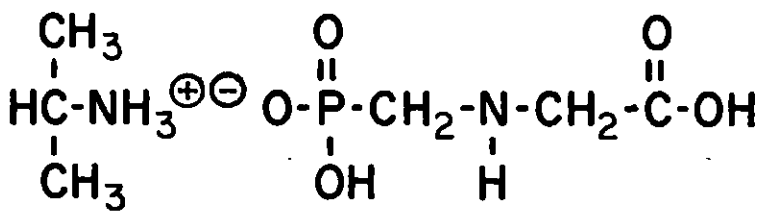


SC-0224

trimethylsulfonium

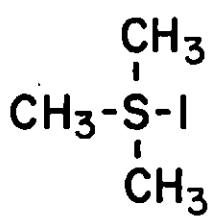
carboxymethylaminomethylphosphonate

C.



Isopropylamine salt of glyphosate

D.



Trimethylsulfonium iodide

Figure 1. Chemical structures of glyphosate, SC-0224, and trimethylsulfonium iodide.

rored that of glyposate although the authors indicated that early season control of horseweed was slightly better with SC-0224 (7, 8, 67). Hutchinson and Banks (35) have reported that, in some cases, bermudagrass clipped at various intervals after treatment with glyphosate demonstrated significantly more regreening than bermudagrass treated similarly with SC-0224.

Metabolic studies with SC-0224 are limited in number. Using isolated soybean mesophyll cells, Bellinder (7) found that glyphosate and SC-0224 both inhibited protein and lipid synthesis but that SC-0224 caused a significantly greater inhibition in both cases. Bellinder (7) also reported that SC-0224 caused greater cellular damage and necrosis in fall panicum. There have also been reports that SC-0224 causes a partial (20%) inhibition of photosystem II activity (the Hill reaction) at a concentration of 0.20 mM compared to no inhibition by glyphosate (Tom Cromartie, Stauffer Chemical Co., personal communication). Other studies have shown that SC-0224 does not inhibit photosynthetic CO_2 fixation indicating that inhibition of photosynthesis is probably not a primary site of action for this herbicide (7).

The structural similarity of glyphosate and SC-0224 as well as the reported similarities and differences in their phytotoxic and metabolic effects have lead to an interest in a more detailed comparison of the mode(s) of action of these herbicides. Studies on the mode of action of glyphosate are many and have centered primarily around the translocation of this compound in plants and the effect of this herbicide on the shikimic acid pathway. Other biochemical effects have been studied as well. Hoagland and Duke (31) have reviewed this topic.

Many previous studies have indicated that glyphosate is translocated in the symplast along the source to sink path of photoassimilates (9, 49, 55, 60, 68) and there is evidence that glyphosate also moves through the apoplast (9, 16, 17, 20, 26, 56). Studies with ^{14}C-labeled glyphosate have shown that it translocates to sites of high metabolic activity including underground vegetative propagules (60). The translocation of foliar-applied glyphosate to underground roots and rhizomes strongly contributes to the efficacy of this herbicide in controlling perennial weeds (5). These translocation properties also correlate well with the finding that early visual symptoms include chlorosis and malformation of growing points. The fact that glyphosate acts slowly compared with other herbicides may be explained by the need for this chemical to be translocated to the plant organs on which it acts most strongly. This slow action may also result from the compound having an initial site of action on a physiological or biochemical process which would kill plants slowly. The shikimic acid pathway is such a process.

In early studies, Jaworski (36) reported that the effects of glyphosate on growth of inflated duckweed (Lemna gibba L.) and Rhizobium japonicum could be partially prevented by supplementing the growth media with the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Based on these data, Jaworski proposed that glyphosate inhibited the biochemical pathway which produces these amino acids — the shikimic acid pathway. Supplemental aromatic amino acids have also been effective in reducing growth inhibition induced by glyphosate in other microorganisms and plant species including Eschericia coli (28, 54), Chlamydomonas reinhardi (28), and in tissue cultures of carrot (Daucus carota L.) (28,

29, 66) and soybean (28). Physiological processes including transpiration (58), protein synthesis (64, 65), and anthocyanin biosynthesis (34) have also been reduced by supplemental aromatic amino acids. However, feeding aromatic amino acids has been ineffective in preventing the glyphosate induced inhibition of growth in tissue cultures of tobacco (Nicotiana tabacum) and soybean (41) and in some intact higher plants including maize (21), soybean (22), and wheat (14).

Several recent studies give strong support to the hypothesis that the primary mechanism of action for glyphosate is the inhibition of the shikimic acid pathway (2, 3, 4, 10, 15, 24, 34, 50, 51, 53, 61, 62, 63). Hollander and Amrhein (34) found that glyphosate prevented the incorporation of ^{14}C-shikimate into the aromatic amino acids in buckwheat (Fagopyrum esculentum Moench). Amrhein et al. (2) found that glyphosate caused the accumulation of shikimate in buckwheat hypocotyls and cultured cells of Galium mollugo L. indicating a blockage of the shikimic acid pathway. Using a cell-free extract of Klebsiella pneumoniae, Steinrucken and Amrhein (62) identified 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP-synthase) as the enzyme of this pathway which is inhibited by glyphosate. Subsequent studies have shown that glyphosate competitively inhibits highly purified EPSP-synthase from K. pneumoniae (4, 61), as well as Neurospora crassa (10) and pea (Pisum sativum L.) (50).

Additional evidence for the importance of the effect of glyphosate on EPSP-synthase activity has come from studies in which microorganisns and plants have become tolerant to glyphosate. Nafziger et al. (51) produced a cell line of carrot which was tolerant to glyphosate by ex-

posing the cell cultures to progressively higher concentrations of glyphosate. The tolerant cell line showed a 52-fold increase in tolerance to the herbicide and 12-fold higher EPSP-synthase activity. The tolerance was effected in this case by a change in the level of enzyme present in the cells as opposed to any change in the effect of glyphosate on a given amount of enzyme. Other studies with cell cultures and bacteria have shown a similar effect (3, 24). A form of EPSP-synthase which has a lower affinity for the herbicide has also been found (15, 25, 63). Researchers have isolated the gene which codes for this altered enzyme and have successfully expressed it in Escherichia coli and tobacco (15, 25). The EPSP-synthase activity in the transformed tobacco plants represented 25% of the total enzyme and these plants demonstrated a significantly increased tolerance to glyphosate (25).

Inhibition of the shikimic acid pathway may lead to decreases in aromatic amino acid pools. Glyphosate has been shown to alter pool levels of free amino acids in various and often conflicting manners. Some have reported decreases in aromatic amino acids and general increases in total free amino acids (32, 36). Others have reported that glyphosate caused general decreases in free amino acid pool levels including the aromatic amino acids (33, 51). Haderlie et al. (29) reported a general increase in amino acid pool levels including phenylalanine.

Decreases in amino acid pools may subsequently lead to decreases in protein synthesis and soluble protein levels. In some studies, there have been attempts to correlate changes in amino acid pools with changes in soluble protein levels. Using axes of dark grown soybean and maize, Hoagland et al. (32, 33) found that, although glyphosate caused decreases

in pool levels of phenylalanine and tyrosine, there was no change in soluble protein levels. Alternatively, Haderlie et al. (29) found that in suspension cultures of carrot, cell protein levels decreased though there was no decrease in phenylalanine levels. Haderlie et al. (29) also found that treatment with glyphosate did not alter the pattern of ^{14}C-leucine and ^{14}C-phenylalanine incorporation into protein. Using rhizome buds of quackgrass and roots of wheat Cole et al. (14) demonstrated that treatment with glyphosate caused preferential incorporation of ^{14}C-phenylalanine versus ^{14}C-leucine suggesting that glyphosate causes a depletion of the phenylalanine pool. This altered incorporation of ^{14}C-phenylalanine occurred concurrently with a depletion of the soluble protein level suggesting that reduction of phenylalanine pools caused a reduction in protein synthesis. Cole et al. (14) suggested that some of the discrepancies listed above may be due to the study of whole plants as opposed to plant parts noted for glyphosate accumulation (e.g. quackgrass rhizome buds) and/or to the presence of different amino acid pools within the plant (14).

Glyphosate has been shown to affect a number of biochemical processes in plants which are not directly associated with the shikimic acid pathway including photosynthesis (57, 60), respiration (52, 60, 64), protein synthesis (14, 64, 65), and chlorophyll biosynthesis (38, 39). Glyphosate has also been shown to stimulate lateral bud growth (6, 45), increase chlorophyll degradation (1, 42), and inhibit ion uptake (11, 23).

In recent studies, glyphosate has been shown to be involved with the metabolism of indole-3-acetic acid (IAA) (41, 43, 44, 46, 47, 48). Glyphosate was found to increase IAA metabolism in a number of species

(43, 44, 47) and decrease the IAA induced ethylene production in tobacco callus (46). High concentrations of IAA were found to partially prevent glyphosate induced inhibition of tobacco and soybean callus growth (41). Lee and Dumas (47) speculate that changes in IAA metabolism may provide the link between the shikimic acid pathway and the many physiological changes caused by glyphosate in plants. They suggest that alterations in phenolic metabolism, which occur as a result of the inhibition of EPSP-synthase, cause changes in IAA metabolism. This hypothesis allows for the variability in the effects of glyphosate on aromatic amino acid pools listed above.

The primary objective of this research was to determine if SC-0224 has a mode of action different from that of glyposate.

Studies were initiated with inflated duckweed to determine the effects of aromatic amino acids on the growth inhibition induced by SC-0224 and to determine the effects of SC-0224 on soluble protein levels, protein synthesis, and the pool levels of free amino acids. Lemna spp. are often used as test plants in physiological studies. Jaworski (36) previously used inflated duckweed in a similar study on the mode of action of glyphosate. Advantages to using this species for this type of study include the rapid rate of growth, decreased biological variation of samples containing many plants, and the ability to grow the plants in sterile culture (13, 37). Because this is an aquatic species without true stems and leaves, penetration and translocation properties important for herbicidal action in terrestrial species may not be represented in studies with inflated duckweed.

Studies were also initiated to determine the effect of SC-0224 on the shikimic acid pathway. This effect was assayed by measuring the conversion of shikimate to anthranilate in a cell-free extract of Klebsiella pneumoniae ATCC 25306. The effect of SC-0224 on the Hill reaction was determined using thylakoids isolated from leaf tissue of 'Alaska' pea.

Glyphosate and trimethylsulfonium halides were included in each of the studies for comparison because the free acid of glyphosate and the trimethylsulfonium ion are the two constituent parts of the SC-0224 structure.

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II. EFFECTS SC-0224 AND GLYPHOSATE ON INFLATED DUCKWEED (Lemna gibba) GROWTH AND EPSP-SYNTHASE ACTIVITY FROM Klebsiella pneumoniae.

Abstract. The effects of the herbicides SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) and glyphosate [N-(phosphonomethyl)glycine] (PMG) on the inhibition of inflated duckweed (Lemna gibba L.) growth and on the conversion of shikimate to anthranilate in a cell-free extract of Klebsiella pneumoniae (ATCC 25306) were compared. Both formulated and technical grade forms of SC-0224 were found to be much more phytotoxic to duckweed than either formulated or technical grade forms of glyphosate. The growth inhibition caused by glyphosate was partially prevented by different combinations of the aromatic amino acids phenylalanine, tyrosine, and tryptophan; whereas, the duckweed growth inhibition caused by SC-0224 could not be reduced by the same amino acid combinations. Trimethylsulfonium ion (TMS), the cationic constituent of the SC-0224 salt, and SC-0224 were found to be equally phytotoxic to duckweed indicating that the phytotoxic effects of TMS may be responsible for differences in the action of glyphosate and SC-0224 on duckweed. SC-0224 and glyphosate equally inhibited the production of anthranilate in the cell-free extract of K. pneumoniae, whereas TMS caused no inhibition. These results indicate that both constituents of the SC-0224 salt, TMS and PMG, are phytotoxic and may act independently.

INTRODUCTION

The herbicide SC-0224 is an experimental, nonselective, postemergence herbicide with potential for use on noncrop areas, in perennial crops, and on reduced tillage cropping systems. Previous laboratory, greenhouse, and field studies have shown SC-0224 to have metabolic and phytotoxic effects very similar to those of glyphosate (3, 4, 7, 17, 24, 39) although differences have occurred in some cases. Bellinder (4) found that glyphosate and SC-0224 caused inhibition of both lipid and protein synthesis in isolated soybean [Glycine max (L.) Merr.] cells; however, SC-0224 caused a significantly greater inhibition in both cases. Bellinder (4) also found that SC-0224 caused greater cellular damage and necrosis in fall panicum (Panicum dicotomiflorum Michx.). Hutchinson and Banks (20) have reported that, in some cases, bermudagrass [Cynodon dactylon (L.) Pers.] clipped at various intervals after treatment with glyphosate showed significantly more regreening than bermudagrass treated similarly with SC-0224.

SC-0224 is the trimethylsulfonium (TMS) salt of N-(phosphonomethyl)glycine (PMG); whereas, glyphosate is commercially formulated as the isopropylamine (IPA) salt of PMG. TMS salts have previously been shown to have growth regulating and phytotoxic effects on a number of plant species including bermudagrass, carpetgrass [Axonopus compressus (Sw.) Beauv.], maize (Zea mays L.), cotton (Gossypium hirsutum L.), pigweed (Amaranthus retroflexus L.) and common duckweed (Lemna minor L.) (32). These species demonstrated

varying degrees of sensitivity to like concentrations of TMS salts; whereas, other species, including radish (Raphanus sativus L.), mustard (Brassica nigra L.), barley (Hordeum vulgaris L.) and soybean were tolerant (32).

The structural similarities of SC-0224 and glyphosate and the observed similarities and differences in the phytotoxic effects of the two compounds has led to an interest in comparing the mechanism(s) of action of these herbicides. Previous studies which have been reviewed by Hoagland and Duke (18) have shown that glyphosate causes a number of biochemical responses in plants including alteration of photosynthesis (30, 33), respiration (28, 33, 36), and reduction of protein (9, 36, 37) and chlorophyll biosynthesis (22, 23). Although some presently believe that glyphosate has other sites of action (14), several recent studies give strong support to the hypothesis that the primary mechanism of action for glyphosate is the inhibition of the shikimic acid pathway (1, 3, 5, 10, 13, 26, 27, 34, 35). Hollander and Amrhein (19) found that glyphosate prevented the incorporation of ^{14}C-shikimate into the aromatic amino acids in buckwheat (Fagopyrum esculentum Moench). Amrhein et al. (1) found that glyphosate caused the accumulation of shikimate in buckwheat hypocotyls and cultured cells of Galium mollugo L. indicating a blockage of the shikimic acid pathway. Using a cell-free extract of Klebsiella pneumoniae, Steinrucken and Amrhein (34) identified 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP-synthase) as the enzyme of this pathway which is inhibited by glyphosate. Subsequent studies have shown PMG to

competitively inhibit highly purified EPSP-synthase from K. pneumoniae (2, 35), as well as Neurospora crassa (5) and pea (Pisum sativum L.) (26). The shikimic acid pathway is responsible for the synthesis of the aromatic amino acids tyrosine, phenylalanine, and tryptophan, as well as many other secondary plant metabolites. Blockage of this pathway may thus lead to decreases in aromatic amino acid pool levels resulting in decreased protein synthesis and subsequent development of biochemical responses such as those listed above.

Partial or complete prevention of glyphosate induced growth inhibition by supplemental aromatic amino acids has been shown in a number of microorganisms and plant species including inflated duckweed (Lemna gibba L.) (21), Rhizobium japonicum (21), Eschericia coli (15, 29), Chlamydomonas reinhardi (15); and in tissue cultures of carrot (Daucus carota L.) (15, 16, 38) and soybean (15). Supplemental aromatic amino acids have also prevented glyphosate induced inhibition of transpiration in bean (Phaseolus vulgaris L.) shoots (31), anthocyanin synthesis in buckwheat (19), and protein synthesis in isolated soybean leaf cells (36, 37). However, the feeding of aromatic amino acids was ineffective in preventing the glyphosate induced inhibition of growth in some intact higher plants including maize (11), soybean (12), and wheat (Triticum aestivum L.) (9) and in tissue cultures of tobacco (Nicotiana tabacum L.) and soybean (25). Different compartmentalization of root-fed versus endogenous amino acids may

be responsible for the failure of aromatic amino acids to prevent growth retardation in these cases (9, 12).

The objectives of this study were to determine the effects of aromatic amino acids on the growth inhibition of inflated duckweed induced by SC-0224, to determine and compare the effects of SC-0224 and the TMS ion on inflated duckweed growth, and to determine the effect of SC-0224 on EPSP-synthase. Glyphosate (PMG) was included in these studies for the purpose of comparison.

MATERIALS AND METHODS

Duckweed Studies

Plant Material. The aquatic flowering plant inflated duckweed (Lemna gibba L. strain G3) was obtained from the collection of Dr. C.F. Cleland of the Smithsonian Radiation Laboratory, Rockville, MD.

Growth Medium. The E medium previously described by Cleland and Gibbs (8) was used in this study. This medium contained per liter: KH_2PO_4, 680 mg; KNO_3, 515 mg; $Ca(NO_3)_2 \cdot 4H_2O$, 1180 mg; $MgSO_4 \cdot 7H_2O$, 500 mg; H_3BO_3, 2.86 mg; $ZnSO_4 \cdot 7H_2O$, 0.22 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.12 mg; $CuSO_4 \cdot 5H_2O$, 0.08 mg; $MnCl_2 \cdot 4H_2O$, 3.62 mg; $FeCl_3 \cdot 6H_2O$, 5.40 mg; tartaric acid, 3.00 mg; EDTA, 9.00 mg; sucrose, 10 g. The medium was adjusted to pH 4.6 with KOH and autoclaved 15 to 20 min at 15 psi.

Chemical Treatments. The chemicals for each of the various treatments were added prior to pH adjustment and autoclaving. Preliminary studies, as well as previous reports (16), determined that autoclaving does not affect the biological activity of the herbicides used in this study. The formulated form of the herbicides were Roundup® in the case of glyphosate and SC-0224 4LC Lot # WDC-1501 in the case of SC-0224. Roundup® is a commercial formulation containing 1 part of the monoisopropylamine salt of glyphosate (4 lb/gal ai) to 0.5 part by weight of MON-0818 surfactant. Technical grade glyphosate (79.0% pure solid) and

technical grade SC-0224 (56.0% pure liquid) were used in this study. Trimethylsulfonium iodide (98.0% pure solid) was obtained from the Aldrich Chemical Co. The isopropylamine salt of glyphosate was prepared by mixing 1 part technical grade glyphosate (2.5 mM) with 1 part isopropylamine hydrochloride (2.5 mM). Isopropylamine hydrochloride was obtained from the Phaltz & Bauer Chemical Co. The amino acids, L-phenylalanine, L-tyrosine, and L-tryptophan, were obtained from the Sigma Chemical Co.

Growth Assays. Growth studies were initiated by inoculating 50 ml of E medium in a 250 ml erlenmeyer flask with one four-frond cluster of Lemna gibba. The flasks were stoppered with cotton plugs to maintain the axenic condition within the flasks, incubated at 29°C, and illuminated continuously under a mixture of fluorescent and incandescent lamps (80 μ Einsteins/ m^2/ sec). These growth conditions were adequate for growth of inflated duckweed to cover the surface of the medium in approximately 10 days. Growth studies ran for 7-day periods after which the plants in each flask were harvested on a fishnet, rinsed with tap water, placed in pre-weighed aluminum trays, and dryed in a drying oven at 55°C for 48 hr. The aluminum trays were then weighed on an analytical balance to determine the dry weight of the tissue.

Enzyme Assay

Organism. A culture of the bacterium Klebsiella pneumoniae (ATCC 25306) was obtained from the American Type Culture Collection, Rockville, MD.

Growth Medium. The organism was grown in the following citric acid-mineral salts medium (34). The following chemicals were dissolved in distilled water to make a final concentration of one liter: $MgSO_4 \cdot 7H_2O$, 200 mg; citric acid$\cdot H_2O$, 2 g; KH_2PO_4-anhydrous, 10 g; $NaNH_4HPO_4 \cdot H_2O$, 3.5 g; glucose, 1.6 g; acid-hydrolysed casein, 50 mg; indole, 2mg. The indole, glucose, and acid-hydrolysed casein were aseptically added to the medium after the other ingredients had been autoclaved for 15 to 20 min at 15 psi. This medium has a pH of about 7.0.

Chemical Treatments. Technical grade glyphosate (79% solid) and technical grade SC-0224 (56.0% liquid) were used in this study. Trimethylsulfonium iodide (98.0% pure solid) was obtained from the Aldrich Chemical Co. Shikimic acid was obtained from the Sigma Chemical Co.

Preparation of Cell-free Extract. Five hundred milliliter plastic erlenmeyer flasks containing 85 ml of the medium described above were inoculated with 10^9 cells from a broth culture shaken for 7 hr (100 rpm, 30°C.). The flasks were then incubated with shaking (100 rpm,

30°C) in a reciprocal shaking bath (Fisher shaking water bath model 127) for 12 to 13 hr and the cell suspension was then centrifuged at 4000 x g for 10 min at 4°C. The pellet was resuspended in 250 ml of 0.9% NaCl and then recentrifuged. The cells of the washed pellet were resuspended in 4 ml of 0.1 M tris/HCl buffer (pH 7.8) per gram fresh weight of cells. The cells of this suspension were disrupted in a French pressure-cell at 16,000 psi and then centrifuged for 30 min at 24,000 x g at 4°C. The supernatant was then frozen until used.

Protein Determination. The protein concentration of the cell-free extract was determined by the Coomasie protein determination method (6). A stock dye solution was prepared by bringing 100 mg of Coomasie G-250 dye, 50 ml of 95% ethanol, and 100 ml of 85% H_3PO_4 to a final volume of 200 ml with distilled H_2O. This stock solution was diluted 1:5 with distilled water and filtered through Whatman # 1 filter paper. Five milliliters of this diluted dye solution were added to a 0.1 ml aliquot of the extract. The absorbance at 595 nm was determined between 2 and 60 minutes after addition of the dye solution to the sample. Maximum absorbance readings are obtained during this time period. The absorbance reading was then compared to a standard protein curve which was prepared using bovine serum albumin.

Assay Procedure. Shikimic acid was converted to anthranilic acid by the enzymes in the cell-free extract (34). A reaction mixture con-

taining tris/HCl buffer (pH 8.2) 30 mM, $MgCl_2$ 5 mM, ribose-5-phosphate 1 mM, NAD 1 mM, glutamine 5 mM, shikimic acid 1 mM, protein 2.5 mg, and the appropriate inhibitor treatments was brought to a final volume of 1 ml and then incubated in test tubes at 37°C for the appropriate time period. All inhibitor solutions were titrated to pH 8.2 prior to addition to the reaction mixture. Anthranilic acid was extracted from the reaction mixture by addition of 0.1 ml of 1 N HCl followed by 4 ml of ethyl acetate. The tubes were mixed with a test tube mixer and then centrifuged at 216 x g for 15 min. The absorbance at 336 nm of the ethyl acetate layer was then measured against an ethyl acetate blank. This absorbance reading was then compared to those of a standard anthranilic acid curve to determine the amount of anthranilate produced during the reaction.

RESULTS AND DISCUSSION

The effects of various concentrations of SC-0224 and glyphosate, in both their technical grades and formulations, on the growth of inflated duckweed, are presented in Table II-1. This comparison shows SC-0224 to be a stronger inhibitor of duckweed growth than glyphosate. Table II-1 also shows that there are no significant differences between the growth inhibition induced by technical grade versus the formulated forms of either herbicide; this finding has two implications. One is that the added constituents of the formulated versus the technical grade forms of the herbicides have no significant growth effects in these cases. The second implication of this comparison is that the use of surfactants as an aid to penetration into the plant is unnecessary in the case of duckweed. This is reasonable in light of the fact that in this study duckweed was floating in continuous contact with the treatment solutions and the chemicals may therefore be taken into the plant either by the roots or by the surface of the fronds.

The effects of supplemental aromatic amino acids on the growth inhibition of duckweed induced by formulated and technical grade glyphosate are presented in Tables II-2 through II-6.

Addition of phenylalanine (0.20 mM) to the growth medium was found to be effective in preventing growth inhibition induced by both the formulated and technical grade forms of glyphosate (0.10 mM)(Table II-2). The addition of both phenylalanine (0.10 mM) and tyrosine (0.10 mM) was more effective than phenylalanine alone in

Table II-1. The effect of various concentrations of formulated (Form.) and technical grade (Tech.) glyphosate and SC-0224 on the dry weight of Lemna gibba grown in E medium (8) for 7 days.

Conc.	Chemical			
	SC-0224 (Tech.)	SC-0224 (Form.)	Glyphosate (Tech.)	Glyphosate (Form.)
(μM)	(mg)[a]			
0	30.12 z	30.12 z	30.12 y	30.12 y
10	10.07 a y	10.96 a y	30.08 b y	29.92 b y
50	3.97 a x	4.47 a x	18.90 b x	18.22 b x
100	1.66 a w	1.33 a w	9.93 b w	10.33 b w

[a]Values represent the average of two experiments with five replications per treatment. The letters a and b correspond to comparisons within rows and the letters w, x, y, and z correspond to comparisons within columns. Means which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table II-2. The effect of phenylalanine on the growth inhibition of Lemna gibba induced by formulated and technical grade glyphosate and SC-0224.

Treatment[a]	Phenylalanine (0.20 mM)	
	-	+
	dry wt (mg)[b]	
Control	27.90 a	29.39 a
Glyphosate[c] (0.10 mM)	10.14 c	16.75 b
Glyphosate[d] (0.10 mM)	11.04 c	19.46 b
SC-0224[c] (0.10 mM)	3.15 d	3.80 d
SC-0224[d] (0.10 mM)	2.53 d	2.13 d

[a]Plants were grown in E medium (8) for 7 days.

[b]Values represent the average of two experiments with five replications per treatment. Means followed by the same letter within the table are not significantly different according to Duncan's multiple range test.

[c]Technical grade herbicide used.

[d]Formulated herbicide used.

Table II-3. The effect of phenylalanine and tyrosine on the growth inhibition of Lemna gibba induced by formulated and technical grade glyphosate and SC-0224.

Treatment[a]	Amino acid supplement (0.10 mM each)	
	None	Phe. + Tyr.[b]
	dry wt (mg)[c]	
Control	31.18 a	33.19 a
Glyphosate[d] (0.10 mM)	10.79 c	24.21 b
Glyphosate[e] (0.10 mM)	10.73 c	22.31 b
SC-0224[d] (0.10 mM)	2.59 d	3.16 d
SC-0224[e] (0.10 mM)	3.37 d	2.77 d

[a]Plants were grown in E medium (8) for 7 days.

[b]Phe. = phenylalanine and Tyr. = tyrosine.

[c]Values represent the average of two experiments with five replications per treatment. Means followed by the same letter within the table are not significantly different according to Duncan's multiple range test.

[d]Technical grade herbicide used.

[e]Formulated herbicide used.

Table II-4. The effect of phenylalanine, tyrosine, and tryptophan on the growth inhibition of Lemna gibba induced by formulated and technical grade glyphosate and SC-0224.

Treatment[a]	Amino acid supplement (0.10 mM each)	
	None	Phe. + Tyr. + Trp.[b]
	dry wt (mg)[c]	
Control	28.42 a	30.89 a
Glyphosate[d] (0.10 mM)	9.61 c	22.76 b
Glyphosate[e] (0.10 mM)	9.99 c	22.76 b
SC-0224[d] (0.10 mM)	3.13 d	3.37 d
SC-0224[e] (0.10 mM)	2.06 d	1.79 d

[a]Plants were grown in E medium (8) for 7 days.

[b]Phe. = phenylalanine, Tyr. = tyrosine and Trp. + tryptophan.

[c]Values represent the average of two experiments with five replications per treatment. Means followed by the same letter within the table are not significantly different according to Duncan's multiple range test.

[d]Technical grade herbicide used.

[e]Formulated herbicide used.

Table II-5. The effect of phenylalanine, tyrosine, and tryptophan on the growth inhibition of Lemna gibba induced by the isopropylamine salt of glyphosate.

Treatment[a]	Chemical conc.	Dry wt[b]
	(mM)	(mg)
Control	----	29.30 a
Phe. + tyr. + trp.[c]	0.10 + 0.10 + 0.10	29.89 a
IPA-glyphosate[d]	0.10	9.70 c
IPA-glyphosate + phe. + tyr. + trp.[c]	0.10 0.10 + 0.10 + 0.10	25.68 b

[a]Plants were grown in E medium (8) for 7 days.

[b]Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different according to Duncan's multiple range test.

[c]Phe. = phenylalanine, tyr. = tyrosine, and trp. = tryptophan.

[d]IPA-glyphosate = isopropylamine salt of glyphosate.

Table II-6. The effect of phenylalanine, tyrosine, and tryptophan on the growth inhibition of Lemna gibba induced by SC-0224 (technical grade) (5.0 μM).

Treatment[a]	Chemical conc.	Dry wt[b]
	(mM)	(mg)
Control	----	29.43 a
Phe. + tyr. + trp.[c]	0.10 + 0.10 + 0.10	29.96 a
SC-0224	0.005	9.39 b
SC-0224 + phe. + tyr. + trp.[c]	0.005 0.10 + 0.10 + 0.10	9.14 b

[a]Plants were grown in E medium (8) for 7 days.

[b]Values represent the average of two experiments with five replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

[c]Phe. = phenylalanine, tyr. = tyrosine, and trp. = tryptophan.

preventing growth inhibition by the two forms of glyphosate (Table II-3). The combination of phenylalanine, tyrosine, and tryptophan was no more effective than the combination of phenylalanine and tyrosine only in preventing growth inhibition of duckweed induced by glyphosate, but was more effective than phenylalanine alone. The data presented in Table II-5 demonstrate that these amino acids were equally effective in preventing growth inhibition induced by the isopropylamine salt of glyphosate without surfactant. Because it was found to be a stronger inhibitor of duckweed growth, SC-0224 was used at 0.05 mM compared with 0.10 mM for glyphosate in these studies. None of the aromatic amino acid treatments were effective in preventing inhibition of duckweed growth by either the technical grade or the formulated form of SC-0224. (Tables II-2 through II-4). When used at 5 μM, SC-0224 caused less growth inhibition than at higher concentrations; however, the amino acid treatments were not effective in reducing growth inhibition at this concentration either (Table II-6).

These supplemental aromatic amino acid studies are in general agreement with the work of Jaworski with glyphosate (21). The failure of the amino acid treatments to prevent growth inhibition caused by SC-0224 indicates that the effect of this herbicide on duckweed is different from that of glyphosate. The fact that SC-0224 is structurally different from glyphosate only in the cationic constituent of its salt leaves two possible explanations for the different effects of the two herbicides. One explanation would be that the TMS ion acts in an additive or synergistic manner when in combination

with PMG to cause the observed differences. A second explanation would be that the TMS ion acts independently of PMG causing the different effects of these herbicides.

The effects of both the TMS and the isopropylamine (IPA) ions on the growth of duckweed are presented in Table II-7. These data show that IPA-hydrochloride causes no significant reduction in duckweed growth; whereas, the TMS salts inhibited duckweed growth. Two different TMS salts were used in this study to account for any effect that the halide anion may have on growth. No significant difference was found between the iodide and the chloride salts of TMS. An additional study (data not shown) showed that potassium iodide (0.10 mM) did not cause growth inhibition of duckweed indicating that the iodide anion did not inhibit duckweed growth at the concentrations used in this study. These data (Table II-7) indicate that the TMS ion alone has strong phytotoxic effects on duckweed growth. A comparison of the effects of SC-0224 and TMS iodide on duckweed growth is presented in Table II-8. These data show that there is no significant difference in the effects of the TMS ion and SC-0224 on duckweed growth. The data of Tables II-7 and II-8 indicate that the differences found between the effects of glyphosate and SC-0224 on duckweed may be accounted for by the effects of the TMS ion acting alone.

The finding that TMS has a stronger and more rapid effect than PMG on inflated duckweed does not preclude the possibility that PMG may also act independently of TMS on the shikimic acid pathway. The effects of TMS, PMG, and SC-0224 on the conversion of shikimate to

Table II-7. The effect of isopropylamine hydrochloride (IPA-HCL), trimethylsulfonium iodide (TMS-I), and trimethylsulfonium chloride (TMS-Cl) on the growth of Lemna gibba in E medium (8) for 7 days.

Treatment	Chemical conc.	Dry wt[a]
	(mM)	(mg)
Control	---	28.59 a
IPA-HCL	0.10	27.30 a
TMS-I	0.10	1.24 b
TMS-Cl	0.10	1.43 b

[a]Values represent the average of two experiments with five replications per treatments. Means within a column which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table II-8. The effects of various concentrations of SC-0224 (technical grade) and trimethylsulfonium iodide (TMS-I) on the dry weight of Lemna gibba grown in E medium (8) for 7 days.

	Chemical treatment	
Conc.	SC-0224	TMS-I
(μM)	(mg)[a]	
0	29.26 a	29.26 a
1	27.49 a	29.99 a
10	10.24 b	9.45 b
50	4.33 c	4.50 c

[a]Values represent the average of two experiments with five replications per treatment. Means within a column which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test. An analysis of variance test showed no significant difference between chemical treatments.

anthranilate in a cell-free extract of Klebsiella pneumoniae were assayed. The reactions of this assay involved four enzymes including shikimate kinase, EPSP-synthase, chorismate synthase, and anthranilate synthase. The results presented in Tables II-9 and II-10 are in agreement with the previous findings of Steinrucken and Amrhein (34) and indicate that SC-0224 like glyphosate is effective in inhibiting these reactions; whereas, TMS-iodide caused no inhibition. Although the enzymes were not assayed individually in the present study, previous studies have shown that of the four enzymes glyphosate inhibits EPSP-synthase exclusively (34). Because of the structural similarities of glyphosate and SC-0224 and the failure of TMS iodide to inhibit these reactions, it is reasonable to assume that SC-0224 was inhibiting EPSP-synthase in this study. Although the enzymes used in this study were from a bacterium, previous studies have shown that glyphosate inhibits EPSP-synthase from other organisms also (5, 10, 13, 26). EPSP-synthase isolated from a higher plant (pea) was found to be more sensitive to PMG than EPSP-synthase isolated from a microorganism (Neurospora crassa) (5, 26). Based on these previously reported findings and the data presented here, it can be assumed that SC-0224 inhibits EPSP-synthase in higher plants.

The data presented here have shown that the two ions of the SC-0224 structure, TMS and PMG, may act independently of each other with each exhibiting phytotoxic effects. These data do not indicate that TMS and PMG act synergistically when used in combination; however, the data do not eliminate possible synergistic action in other

Table II-9. The effects of glyphosate (PMG) (1 mM), SC-0224 (1 mM), and trimethylsulfonium iodide (TMS-I) (1 mM) on the conversion of shikimate to anthranilate in a cell-free extract of Klebsiella pneumoniae after various time intervals.

	Treatment[a]			
Time	Control	PMG	SC-0224	TMS-I
(min)	(µmol anthranilate)[b]			
0	45 b u	48 b u	49 b u	43 b u
15	65 a v	42 b u	43 b u	58 a v
30	75 a w	48 b u	38 b u	88 a w
40	120 a x	40 b u	45 b u	115 a x
60	131 a y	50 b u	49 b u	120 a y
120	183 a z	52 b u	60 b u	190 a z

[a]Technical grade herbicides were used in this experiment.

[b]Values represent the average of two experiments with three replications per treatment. The letters a and b correspond to comparisons within rows and the the letters u through z correspond to comparisons within columns. Means which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table II-10. The effects of various concentrations of glyphosate (PMG), SC-0224, and trimethylsulfonium iodide (TMS-I) on the conversion of shikimate to anthranilate in a cell-free extract of Klebsiella pneumoniae after 40 min.

	Treatment[a]		
Conc.	PMG	SC-0224	TMS-I
(μM)	(μmol anthranilate)[b]		
0	120 z	120 z	120 x
1	83 a y	90 a y	125 b x
10	50 a x	53 a x	135 b x
100	40 a x	45 a x	115 b x

[a]Technical grade herbicides were used in this experiment.

[b]Values represent the average of two experiments with three replications per treatment. The letters a and b correspond to comparisons within rows and the letters x, y, and z correspond to comparisons within columns. Means which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

plant species and/or on other physiological processes. SC-0224, like glyphosate, was found to be a strong inhibitor of the shikimic acid pathway. Inhibition of this pathway is believed to be the primary mechanism of action for PMG, and this study has indicated that use of PMG as its TMS salt (SC-0224) does not alter its ability to inhibit this pathway. The present study has also indicated that the phytotoxic effect of the TMS ion is more important than that of PMG in the case of duckweed, an aquatic species. However, this relationship may not hold in the case of terrestrial species which may be less sensitive to TMS.

Glyphosate and SC-0224 have been shown to have similar efficacy in many field studies (3, 4, 17, 24, 39), which suggests that PMG is the most active of the two SC-0224 ions in these terrestrial species. However, Hutchinson and Banks (20) have reported that, in some cases, bermudagrass is more sensitive to SC-0224 than to glyphosate. These similarities and differences in the action of glyphosate and SC-0224 may be due to differences in the sensitivity of various species to TMS. Previous studies (32) have found TMS to be much more phytotoxic to bermudagrass than to many other plant species. These findings suggest that in some plant species PMG may be the most phytotoxic ion of the SC-0224 structure, whereas, in other species TMS may be the most active ion.

In conclusion, SC-0224 contains two constituent parts which apparently act independently in the systems studied here. TMS is apparently the most important of the two ions in the case of duckweed exerting a more acute phytotoxic response which overshadows the

slower effect on the shikimate pathway. However this situation may be reversed in the case of many terrestrial species possibly due to differences in the phytotoxicity of TMS among species and/or differences in the penetration and translocation properties of TMS in terrestrial species. Additional studies in which SC-0224 is compared directly with TMS and glyphosate on terrestrial species are needed to resolve this situation conclusively.

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III. EFFECTS OF SC-0224 AND GLYPHOSATE ON FREE AMINO ACIDS, SOLUBLE PROTEIN, AND PROTEIN SYNTHESIS IN INFLATED DUCKWEED (Lemna gibba).

Abstract. The effects of the herbicides SC-0224 (trimethysulfonium carboxymethylaminomethylphosphonate) and glyphosate [N-(phosphonmethyl)glycine] (PMG) on the pool levels of free amino acids, soluble protein and protein synthesis in inflated duckweed (Lemna gibba L.) were compared. SC-0224 caused larger increases than glyphosate in the pool levels of amino acids; the increases caused by SC-0224 were similar, however, to those caused by trimethylsulfonium iodide (TMS-I). Expressed on a per gram fresh weight basis none of the chemical treatments caused significant changes in soluble protein or the incorporation of ^{14}C-leucine into soluble protein. On a per flask basis (allowing for decreased growth in treated flasks), both herbicides and TMS-I caused significant decreases in soluble protein and ^{14}C-leucine incorporation. SC-0224 and TMS-I caused larger decreases than glyphosate in both cases but the SC-0224 and TMS-I treatments were not significantly different. These data indicate that differences in the phtotoxicity of SC-0224 and glyphosate may be due to the action of the trimethylsulfonium ion of the SC-0224 structure. Possible causes for the observed changes in amino acid pools are discussed.

INTRODUCTION

The herbicide SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) is an experimental, nonselective, postemergence herbicide with potential for use on non-crop areas, in perennial crops, and in reduced tillage cropping systems. Previous laboratory, greenhouse, and field studies have shown SC-0224 to have metabolic and phytotoxic effects very similar to those of glyphosate [N-(phosphonomethyl)glycine] (PMG) (4, 5, 10, 18) although differences have occured in some cases (5, 23). Glyphosate is commercially formulated as the isopropylamine salt of PMG; whereas, SC-0224 is the trimethylsulfonium (TMS) salt of PMG. Other TMS salts have previously been shown to have growth regulating and phytotoxic effects on a number of plant species (29). Therefore, the TMS ion may contribute to differences in the phytotoxicity of glyphosate and SC-0224. The structural similarities of SC-0224 and glyphosate and the observed similarities and differences in the phytotoxic effects of the two compounds have led to an interest in comparing the mechanism(s) of action of these herbicides.

Glyphosate has previously been shown to affect plant metabolism in a number of ways including alterations in protein synthesis (15, 16, 36, 37), and the pool levels of free amino acids (14, 17, 20, 21, 24, 26, 27) and soluble protein (12, 17). Many recent studies have given strong support to the hypothesis that inhibition of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate-synthase (EPSP-synthase) is the primary site-of-action

of glyphosate and that other metabolic effects are secondary in nature (1, 2, 13, 19, 22, 26, 30, 31). According to this hypothesis, the inhibition of EPSP-synthase may lead to decreased levels of aromatic amino acids which subsequently cause an inhibition of protein synthesis.

However, there have been conflicting reports on the effects of glyphosate on pool levels of free amino acids and soluble protein. Some have reported a decrease in aromatic amino acids and a general increase in total free amino acids (20, 24, 27). Others (21, 26) have reported that glyphosate caused general decreases in free amino acid pool levels including the aromatic amino acids. Haderlie et al. (17) reported a general increase in amino acid pool levels including phenylalanine.

In some studies, attempts were made to correlate changes in amino acid pool levels with changes in soluble protein pool levels (12, 17, 20, 21). Using axes of dark grown soybeans [Glycine max (L.) Merr.] and maize (Zea mays L.), Hoagland et al. (20, 21) found that, although glyphosate caused decreases in pool levels of phenylalanine and tyrosine, there was no change in soluble protein levels. Alternatively, Haderlie et al. (17) found that in suspension cultures of carrot (Daucus carota L.) cell protein levels decreased though there was no decrease in phenylalanine levels. Haderlie et al. (17) also found that treatment with glyphosate did not alter the pattern of ^{14}C-leucine and ^{14}C-phenylalanine incorporation into protein. Using rhizome buds of quackgrass [Agropyron repens (L.) Beauv.] and roots of wheat (Triticum aestivum L.), Cole et al. (12)

demonstrated that treatment with glyphosate caused preferential incorporation of ^{14}C-phenylalanine versus ^{14}C-leucine suggesting that glyphosate causes a depletion of the phenylalanine pool. This depletion of phenylalanine occurred concurrently with a depletion of the soluble protein level suggesting that reduction of phenylalanine pools caused a reduction in protein synthesis. Cole et al. (12) suggested that some of the discrepancies listed above may be due to the study of whole plants as opposed to plant parts noted for glyphosate accumulation (e.g. quackgrass rhizome buds) and/or to the presence of different amino acid pools within the plant.

Gianfagna (16) found that in Lemna perpusilla L. glyphosate caused reductions in both soluble protein levels and the incorporation of ^{14}C-leucine into protein. Tymonko (36) found that glyphosate inhibited protein synthesis and reduced soluble protein levels in isolated soybean cells. Phenylalanine and tyrosine were effective in reducing the inhibition of protein synthesis and in bringing soluble protein levels back to the control level (36, 37).

Previous studies on the metabolic action of SC-0224 are limited. Using isolated soybean leaf cells, Bellinder found that SC-0224 gave greater inhibition of lipid synthesis and protein synthesis than did glyphosate (5).

The objectives of this study were to compare the effects of SC-0224, glyphosate, and trimethylsulfonium iodide on the pool levels of free amino acids, soluble protein levels and on the incorporation of ^{14}C-leucine into soluble protein.

MATERIALS AND METHODS

Plant Material. The aquatic flowering plant inflated duckweed (Lemna gibba L. strain G3) was obtained from the collection of Dr. C.F. Cleland of the Smithsonian Radiation Laboratory, Rockville, MD.

Growth Medium. The E medium previously described by Cleland and Gibbs (11) was used in this study. This medium contained per liter: KH_2PO_4, 680 mg; KNO_3, 515 mg; $Ca(NO_3)_2 \cdot 4H_2O$, 1180 mg; $MgSO_4 \cdot 7H_2O$, 500 mg; H_3BO_3, 2.86 mg; $ZnSO_4 \cdot 7H_2O$, 0.22 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.12 mg; $CuSO_4 \cdot 5H_2O$, 0.08 mg; $MnCl_2 \cdot 4H_2O$, 3.62 mg; $FeCl_3 \cdot 6H_2O$, 5.40 mg; tartaric acid, 3.00 mg; EDTA, 9.00 mg; sucrose, 10 g. The medium was adjusted to pH 4.6 with KOH and autoclaved 15 to 20 min at 15 psi.

Chemical Treatments. The chemicals for each of the various treatments were added prior to pH adjustment and autoclaving. Preliminary studies, as well as previous reports (17) determined that autoclaving does not affect the biological activity of the herbicides used in this study. The treatment chemicals used in this study included technical grade SC-0224 (56.0% pure liquid), technical grade glyphosate (79.0% pure solid) and trimethylsulfonium iodide (98.0% solid).

Preparation of Tissue for Extraction. Studies were initiated by inoculating 50 ml of E medium in a 250 ml erlenmeyer flask with one four-frond cluster of inflated duckweed. The flasks were stoppered

with cotton plugs to maintain the axenic condition, incubated at 29°C, and illuminated continuously under flourescent and incandescent lamps (80 μ Einsteins/ m^2/ sec.). These growth conditions were adequate to allow growth of the inflated duckweed to cover the surface of the medium in approximately 10 days. After 7 days, the plants in each flask were aseptically transferred to another 250 ml flask containing 50 ml of E medium and the appropriate chemical treatment. After an additional 48 hr, the plants in each flask were harvested, rinsed with distilled water, gently blotted dry with paper towels, weighed on an analytical balance to determine the fresh weight, and then extracted as dictated by the specific experiment.

Amino Acid Extraction and Determination. The tissue was ground in liquid nitrogen with a mortar and pestle and homogenized with a ground glass piston homogenizer in hot 80% ethanol. The ethanol was removed with a flash evaporator and the remaining aqueous portion of the sample was extracted three times with equal volumes of chloroform (24). The concentrations of individual amino acids were then determined with a Beckman Model 21 Automatic Amino Acid Analyzer (25).

Protein Extraction and Determination. The tissue was homogenized in a ground glass piston homogenizer in 0.1 M tris/HCl buffer (pH 7.5) and this mixture was centrifuged for 30 min at 24,000 x g at 4°C. The protein concentration of the supernatant was determined by the Coomasie protein determination method (8). A stock dye solution was

prepared by adding to a final volume of 200 ml: 100 mg of Coomasie G-250 dye, 50 ml of 95% ethanol, 100 ml of 85% H_3PO_4 and distilled H_2O. This stock solution was diluted 1:5 with distilled water and filtered through Whatman # 1 filter paper. Five milliliters of this diluted dye solution was added to a 0.1 ml aliquot of the extract. The absorbance at 595 nm was determined between 2 and 60 min after addition of the dye solution to the sample. Maximum absorbance readings are obtained during this time period. The absorbance reading was then compared to a standard protein curve which was prepared using bovine serum albumin.

Incorporation of L-^{14}C-leucine Into Soluble Protein. Inflated duckweed was grown and prepared for extraction as described above. Forty hours after transfer of the plants to treatment solutions, 2 µCi of L-^{14}C-leucine (specific activity 310 µCi/mmole) were added to the flasks. After an additional 8 hr (48 hr after transfer) the plants were harvested. The plants were homogenized in a ground glass piston homogenizer in 0.1 M tris/HCl buffer (pH 7.5) and a 0.1 ml aliquot was removed to determine the uptake of radioactivity into the plants. The remaining homogenate was centrifuged for 30 min at 24,000 x g and 4°C. To precipitate the protein in the supernatant, cold trichloroacetic acid (TCA) was added to equal 5% (w/v). After 20 min the precipitate was collected by centrifuging for 10 min at 3,000 x g and 4°C. The pellet was resuspended in 5% (w/v) TCA and centrifuged again. The final pellet was dissolved in ScintiVerse E scintillation cocktail (Fischer Scientific Co.) and the radioactiv-

ity was determined with a Beckman LS-250 liquid scintillation counter.

RESULTS AND DISCUSSION

The effects of glyphosate, SC-0224, and trimethylsulfonium iodide on the growth and percent water content of duckweed during the 48 hr post-treatment period described in the materials and methods are presented in Table III-1. These results show significant differences in fresh weight gain and percent water content among the treatments. The dry weights were not significantly different although the trends were similar to those of the fresh weight and water content data. Because of such differences in fresh weight gain and water content, cellular contents may be more concentrated in treated plants compared with the control. The concentration of amino acid and protein pools may thus give a false impression of their rate of synthesis when expressed on a per gram fresh weight basis. The data of this study are, therefore, expressed on a per flask basis as well as a per gram fresh weight basis.

The effects of glyphosate, SC-0224, and TMS-I on the pool levels of free amino acids are presented in Tables III-2 and III-3. The initial objective of this study was to compare the effects of the herbicides on aromatic amino acids. A sodium based buffer system was used because it was available and adequate to resolve phenylalanine and tyrosine although tryptophan is not resolved on this system. Also because glutamine and asparagine elute at similar times with serine, threonine and glutamate, these amino acid concentrations must be reported as a lump sum rather than individually.

Table III-1. The effects of glyphosate, SC-0224, and trimethylsulfonium iodide (TMS-I) on growth and water content of Lemna gibba during the 48 hr post-treatment time period.

Chemical	Fresh wt[a]	Dry wt[ab]	H_2O[a]
	(mg)	(mg)	(%)
Control	586.1 a	37.7	93.6 a
Glyphosate	496.1 b	36.7	92.6 b
SC-0224	379.2 c	34.1	91.0 c
TMS-I	385.1 c	33.9	91.2 c

[a]Values represent the average of three experiments with four replications per treatment. Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test. Analysis of variance showed no significant difference among dry weight means.

[b]The average dry weight at the beginning of the 48 hr treatment period was 23.5 mg.

Table III-2 The effects of glyphosate (PMG), SC-0224, and trimethylsulfonium iodide (TMS-I) on the pool levels of free amino acids in Lemna gibba expressed on per gram fresh weight basis.

Amino acid	Chemical treatment			
	Control	PMG	SC-0224	TMS-I
	(nmol/g fresh wt)[a]			
Arginine	73.1 b	460.2 ab	729.3 a	776.1 a
Aspartate	985.6 a	1423.9 a	923.6 a	1103.7 a
Glycine	45.4 a	62.1 a	78.3 a	78.8 a
Alanine	358.1 b	928.1 b	4045.0 a	3588.0 a
Cysteine[b]	-	-	-	-
Valine	70.3 b	111.2 b	276.1 a	282.5 a
Isoleucine	18.6 c	41.0 bc	59.9 ab	73.9 a
Leucine	44.9 b	45.8 b	71.2 ab	79.0 a
Tyrosine	11.5 c	35.9 b	42.4 b	77.1 a
Phenylalanine	22.3 b	48.8 a	18.6 b	13.6 b
Mixture[c]	2087.9 b	6683.8 a	8789.1 a	9752.4 a

[a]Values represent the average of two experiments with three replications per treatment. Means within a row which are followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

[b]Below detection limits.

[c]Values represent the total of the pool levels for threonine, serine, glutamate, asparagine, and glutamine.

Table III-3. The effects of glyphosate (PMG), SC-0224, and trimethylsulfonium iodide (TMS-I) on the pool levels of free amino acids in Lemna gibba expressed on a per flask basis.

Amino acid	Chemical Treatment			
	Control	PMG	SC-0224	TMS-I
	(μmol/flask)[a]			
Arginine	42.8 b	228.3 a	276.6 a	298.9 a
Asparate	577.7 ab	706.4 a	350.3 c	425.0 bc
Glycine	26.6 a	30.8 a	29.7 a	30.4 a
Alanine	209.9 b	460.4 b	1533.9 a	1381.8 a
Cysteine[b]	-	-	-	-
Valine	41.2 b	55.2 b	104.7 a	108.8 a
Isoleucine	10.9 b	20.3 ab	22.7 a	28.5 a
Leucine	26.3 a	22.7 a	27.0 a	30.4 a
Tyrosine	6.8 c	17.8 b	16.1 b	29.7 a
Phenylalanine	13.1 b	24.2 a	7.0 bc	5.2 b
Mixture[c]	1223.7 b	3315.8 a	3332.8 a	3755.6 a

[a]Values represent the average of two experiments with three replications per treatment. Means within a row are not significantly different at the 0.05 level according to Duncan's multiple range test.

[b]Below detection limits.

[c]Value represents the total of the pool levels for threonine, serine, glutamate, asparagine, and glutamine.

On a per gram fresh weight basis (Table III-2) glyphosate caused significant differences (increases) from the control in pool levels of only phenylalanine and tyrosine. On a per flask basis (Table III-3) the magnitudes of difference were changed but significant differences remained the same for all of the amino acids with the exception of arginine which was also significantly greater than the control. There was a general trend toward increased pool levels in the glyphosate treatment, although other amino acid concentrations were not significantly different from the control. The increased levels of phenylalanine and tyrosine are inconsistent with the previous findings of Jaworski (24) and with the hypothesis that reduction in aromatic amino acid pools leads to the many other physiological effects of glyphosate. Free amino acid pools, however, are known to vary considerably in plants being affected by such factors as stage of growth, nitrogen nutrition, photoperiod, and mineral nutrition (3). Haderlie et al. (17) found that glyphosate caused no change in aromatic amino acid concentration in carrot cell cultures after 72 hr but phenylalanine began to increase at 96 hr. The presence of separate intracellular amino acid pools may contribute to this variability (3, 5, 6, 12, 28).

On a per gram fresh weight basis (Table III-2), SC-0224 caused increases significantly different from the control in pool levels of arginine, alanine, valine, isoleucine, and tyrosine. On a per flask basis (Table III-3), the concentration of aspartate was significantly less than the control with all other comparisons remaining the same. Changes caused by TMS-I were not significantly

different from those caused by SC-0224 with the exception of tyrosine which was significantly greater in the TMS-I treatment.

The similarities between the SC-0224 and TMS-I treatments in comparison to the control and the glyphosate treatment indicate that the TMS ion may cause the differences in the phytotoxicity of SC-0224 and glyphosate on duckweed. On the other hand, the fact that SC-0224 and TMS-I have a different effect on tyrosine only, may be an indication that the N-(phosphonomethyl)glycine portion of the SC-0224 structure was also active on the shikimic acid pathway in this treatment.

Possible explanations for the observed increases in the free amino acid pool levels include the inhibition of protein synthesis, increased protein hydrolysis, decreased use of amino acids as respiratory carbon sources, and/or increased amino acid biosynthesis (3, 7). To determine the effects of these treatment chemicals on protein metabolism, soluble protein levels and incorporation of ^{14}C-leucine into soluble protein were determined. A decrease in soluble protein concentration may indicate a decrease in protein synthesis and/or an increase in protein hydrolysis. A decrease in the incorporation rate of ^{14}C-leucine into soluble protein should indicate a decrease in protein synthesis only since the time period of this assay (8 hr) is probably not long enough to allow a complete turnover of protein. Although stress conditions are known to increase protein turnover rates, the protein half-life in common duckweed (Lemna minor L.) has been reported to be two days under stress conditions (35).

The effects of glyphosate, SC-0224, and TMS-I on soluble protein levels in inflated duckweed are presented in Table III-4. On a per gram fresh weight basis, there were no significant differences among treatments; however, on a per flask basis there were significant differences showing the SC-0224 and TMS-I treatments to have a lower protein content than either glyphosate or the control. results of the ^{14}C-leucine study are presented in Table III-5 and these results are similar to those of the soluble protein study. There was no significant difference among treatments in the uptake of radioactivity into the plants (Table III-6). The results of these studies, when expressed on a per gram fresh weight basis, are consistent with the hypothesis that inhibition of protein synthesis triggers the observed increases in amino acid pool levels. However, if changes in these processes decrease only in response to decreases in growth related processes, the per gram fresh weight data may more accurately represent the effect of these chemicals on protein metabolism.

SC-0224 and TMS-I may induce a senescence response in inflated duckweed leading to the observed changes in amino acid and protein metabolism. Others have previously suggested that metabolic and ultrastructural changes caused by glyphosate indicate that this herbicide induces senescence in plants (9, 27). Protein hydrolysis leading to the accumulation of free amino acids, particularly glutamine and asparagine, has been observed in senescing leaves in a number of plant species (3, 32, 33, 34). Reduction of protein synthesis and respiration are also senescence events and could lead

Table III-4. The effects of glyphosate, SC-0224, and trimethylsulfonium iodide (TMS-I) on the soluble protein level in Lemna gibba.

Chemical	Soluble protein	
	(mg/g fresh wt)[a]	(mg/flask)[b]
Control	9.30	5448.4 a
Glyphosate	9.54	4732.6 b
SC-0224	9.43	3674.0 c
TMS-I	9.54	3576.0 c

[a]Values represent the average of two experiments with three replications per experiment. Analysis of variance showed no significant difference at the 0.05 level.

[b]Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table III-5. The effects of glyphosate, SC-0224, and trimethylsulfonium iodide (TMS-I) on the uptake of ^{14}C-leucine into Lemna gibba

Chemical	Uptake of radioactivity
	DPM/g fresh wt[a]
Control	16,804
Glyphosate	16,052
SC-0224	17,063
TMS-I	17,252

[a]Values represent the average of two experiments with three replications per treatment. Analysis of variance showed no significant difference among means at the 0.05 level.

Table III-6. The effects of glyphosate, SC-0224, and trimethylsulfonium iodide (TMS-I) on the incorporation of ^{14}C-leucine into soluble protein in Lemna gibba

Chemical	Radioactivity incorporated[a]	
	DPM/g fresh wt[b]	DPM/flask[c]
Control	5881	34,470 a
Glyphosate	5723	28,392 b
SC-0224	5624	21,328 c
TMS-I	5237	20,169 c

[a]Values represent the average of two experiments with three replications per treatment.

[b]Analysis of variance showed no significant difference among means at the 0.05 level.

[c]Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

to the increase of free amino acids observed in this study. The results presented in this study are consistent with the responses expected of senescensing plants; however, additional work is needed to confirm this hypothesis. These studies could include a more detailed study of the effects of these herbicides on protein and amino acid metabolism in combination with studies on other known effects of senescense. These effects include decreased respiration, increased chlorophyll degradation, altered growth regulator concentration and ultrastructrual changes (32).

An increase in amino acid biosynthesis seems to be a less likely explanation for the changes observed in this study due to the fact that less anabolic reactions would be expected as a response to treatment with growth inhibiting chemicals. However, blockage of one pathway may shunt the metabolic precursors from that pathway to a second pathway. Examination of Tables III-2 and III-3 reveals that the amino acids which showed the largest increases in the SC-0224 and TMS-I treatments were alanine followed by valine. Since alanine and valine derive their carbon skeletons from pyruvate (3), it is possible that increases in these amino acids are due to an inhibition of a pathway which has pyruvate as a precursor (e.g. the Kreb's cycle).

The data presented in this study have shown that SC-0224 and TMS-I caused changes in the pool levels of some free amino acids in inflated duckweed. These changes were also significantly different from those caused by glyphosate in most instances. On a per flask basis, SC-0224 and TMS-I were also found to have stronger effects

than glyphosate on soluble protein levels and ^{14}C-leucine incorportation in soluble protein. These results thus indicate that SC-0224 has a different mode of action than glyphosate on inflated duckweed but that the difference may be attributed to stronger effects of the TMS ion masking the effects of the PMG portion of the SC-0224 structure. The relative toxicities of PMG and TMS found in this study on an aquatic species may not hold in the case of all species as suggested by the previous findings that several terrestrial species have different levels of sensitivity to TMS salts (29). These differences may be due to different requirements for penetration and translocation in terrestrial species. The data are not inconsistent with any of the stated possible explanations for the observed changes in free amino acid pools and it is possible that all of these factors contribute partially to the observed changes. Additional research with other plant species is needed to gain a further understanding of the differences in the modes of action of SC-0224 and glyphosate.

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IV. THE EFFECTS OF SC-0224 ON PHOTOSYNTHETIC ELECTRON TRANSPORT.

Abstract. The effects of the herbicide SC-0224 on photosynthetic electron transport (the Hill reaction) was determined using isolated thylakoids from Alaska pea (Pisum sativum L.). The action of SC-0224 was compared with the action of glyphosate [N-(phosphonomethyl)-glycine], trimethylsulfonium iodide (TMS), and diuron [3-(3,4-dichorophenyl)-1,1-dimethylurea]. SC-0224, glyphosate and TMS-I did not inhibit the Hill reaction at concentrations up to 10 mM; whereas, diuron caused an almost total inhibition at 0.10 mM. The results of this study indicate that SC-0224 is not an inhibitor of photosynthetic electron transport.

INTRODUCTION

Previous studies with the herbicide glyphosate [N-(phosphonomethyl)glycine] have indicated that the effects of this herbicide on photosynthesis are probably secondary in nature (4, 6, 9, 11,). Glyphosate (5 mM) caused a 25% inhibition of CO_2 fixation in isolated soybean [Glycine max (L.) Merr.] leaf cells after a 20 hr exposure period (11); however, the time period required to obtain this level of inhibition does not indicate that inhibition of photosynthesis is a primary site of action for this herbicide. In quackgrass [Agropyron repens (L.) Beauv.] glyphosate caused a reduction of photosynthesis only after 72 hr and the authors concluded that photosynthesis is probably not a primary mode of action of the compound (9). Glyphosate caused a reduction in net photosynthesis in bean (Phaseolus vulgaris L.) after only 6 hr but the authors concluded that this effect resulted from decreased leaf conductance. Previous studies have also indicated that glyphosate does not inhibit the Hill reaction (4, 6).

SC-0224 (trimethylsulfonium carboxymethylaminomethylphosphonate) is an experimental herbicide which is structurally similar to glyphosate. Glyphosate is commercially formulated as the isopropylamine salt of N-(phosphonomethyl)glycine (PMG); whereas, SC-0224 is the trimethylsulfonium salt of PMG. Previous laboratory and field studies have shown SC-0224 to have phytotoxic and metabolic effects similar to those of glyphosate (1, 2, 12), although differences have occurred in some cases (1). SC-0224 was found to have

no significant effect on CO_2 fixation in isolated soybean cells (1); however, there have been reports of SC-0224 causing partial inhibition of photosynthetic electron transport (Tom Cromartie, Stauffer Chemical Co., personal communication).

It was the purpose of this study to compare the effects of PMG, SC-0224, and trimethylsulfonium iodide on the Hill reaction.

MATERIALS AND METHODS

The effects of SC-0224, glyphosate, and trimethylsulfonium iodide on photosynthetic electron transport were assayed by the methods of Richard et al. (6) with minor modifications.

Plant Material. Alaska pea (Pisum sativum L.) was germinated and grown in vermiculite in a glass house for 14 days. Thylakoids were isolated from leaf tissue of the 14 day old seedlings by the procedures outlined below.

Preparation of Solutions.

Grinding medium: 0.10 M tricine buffer (pH 7.8); 0.40 M sorbitol; 10 mM NaCl; 50 mM sodium ascorbate; 0.25% (w/v) bovine serum albumin.

Hypoosmotic medium: 10 mM NaCl.

Reaction medium A: 0.90 mM tricine buffer (pH 7.8); 180 mM sorbitol; 9.0 mM NaCl; 9.0 mM $MgCl_2$; 0.80 mM NH_4Cl.

Reaction medium B: reaction medium A plus 1.60 mM methyl viologen; 1.60 mM NaN_3.

Treatment solutions: Timethylsulfonium iodide (98.0% pure solid, Aldrich Chemical Co.), SC-0224 (technical grade 56.0% pure liquid), and glyphosate (technical grade 79.0% pure solid) were dissolved in

reaction medium B at appropriate concentrations prior to pH adjustment.

Preparation of Thylakoids. Tissue (10 g) was homogenized in a Sorval Omni Mixer for 10 sec in 100 ml of grinding medium (0°C) and filtered through Miracloth. The filtrate was kept on ice and in darkness as much as possible through the following steps. The filtrate was centrifuged for 4 min at 500 x g and 4°C and the supernatant was recentrifuged for 7 min at 1,000 x g and 4°C. The pellet from the second centrifugation was resuspended in 100 ml of hypoosmotic medium to disrupt the chloroplasts. This suspension was then centrifuged for 10 min at 1,000 x g and 4°C and the pellet was resuspended in reaction medium A. This suspension was diluted to equal 40 µg/ml chlorophyll. Chlorophyll was determined by diluting 1.0 ml of the thylakoid suspension in 19 ml of 80% (v/v) acetone and then measuring the absorbance at 652 nm. The chlorophyll concentration was calculated by the following formula: $A_{(652)} \times 0.58 =$ chlorophyll mg/ml (7).

Electron Transport Assays. One milliliter of reaction mixture B with the appropriate treatment chemical concentration was added to 1 ml of the thylakoid suspension. The effects of the chemical treatments on the Mehler reaction (O_2 uptake) was then measured with a Clark Type oxygen electrode (3).

RESULTS AND DISCUSSION

The effects of PMG, SC-0224, and trimethylsulfonium iodide (TMS-I) on whole chain photosynthetic electron transport (the Hill reaction) are presented in Tables I and II. TMS-I was included to test any possible individual effect that the cationic constituent of the SC-0224 structure (the trimethylsulfonium ion) might have on electron transport. Previous studies have shown that isopropylamine stimulated electron transport rates (6); therefore, glyphosate was used as the free acid in this study. With no prior incubation, there was no significant inhibition of photosynthetic electron transport at rates up to 10 mM (Table I). Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], a known inhibitor of these reactions, was included as a standard and caused total inhibition of electron transport at 0.01 mM. Similar results were obtained when the chloroplasts were preincubated with the treatment solution for 20 min (Table II).

As mentioned in the introduction, previous results have indicated that SC-0224 partially inhibited photosystem II activity measured by the reduction of dichlorophenol indophenol (DCPIP). In that study, SC-0224 at 0.20 mM showed 20% inhibition of photosystem II activity; whereas, glyphosate, TMS-I, and trimethylsulfonium chloride did not inhibit the reactions (Tom Cromartie, Stauffer Chemical Co., personal communication). The results of the present study differ from those previously reported. The results of the present study do not necessarily contradict those of the earlier

Table IV-1. The effects of glyphosate, SC-0224, trimethylsulfonium iodide, and diuron on the Mehler reaction of photosynthesis.

Chemical	Conc.	Rate[a]
	(mM)	(μmol O_2/mg chlorophyll/hr)
Control	0	79 a
Glyphosate	10.0	85 a
	5.0	86 a
	1.0	81 a
	0.1	79 a
SC-0224	10.0	80 a
	5.0	78 a
	1.0	79 a
	0.1	82 a
TMS-I	10.0	79 a
	5.0	78 a
	1.0	82 a
	0.1	78 a
Diuron	0.1	4 b

[a]Values represent the average of five experiments with three replications per treatment. Means which are followed by the same letter are not significantly different according to Duncan's multiple range test.

Table IV-2. The effects of glyphosate, SC-0224, trimethylsulfonium iodide, and diuron on the Mehler reaction of photosynthesis after a 20 min dark incubation.

Chemical	Conc.	Rate[a]
	(mM)	(μ mol O_2/mg chlorophyll/hr)
Control	0	77 a
Glyphosate	10.0	73 a
	5.0	80 a
SC-0224	10.0	73 a
	5.0	80 a
TMS-I	10.0	77 a
	5.0	74 a
Diuron	0.1	2 b

[a]Values represent the average of five experiments with three replications per treatment. Means which are followed by the same letter are not significantly different according to Duncan's multiple range test.

study because the inhibition in the DCPIP study was only partial and the method was more sensitive than the method here.

In assessing the significance of these data, several facts need to be considered. The method used in the present study is adequate for identifying photosynthetic inhibitors, although it may be less sensitive than DCPIP reduction (10). The concentrations of SC-0224 used in this study were relatively high when compared with concentrations of photosynthetic inhibitors such as diuron needed to inhibit electron transport. SC-0224 does not have the structural moiety common for most Hill reaction inhibitors, ie. an electron deficient sp^2 carbon atom adjacent to a nitrogen atom with a free pair of electrons (5). Also, SC-0224 was found not to inhibit CO_2 fixation (2).

In conclusion, the data presented here as well as the other facts listed above indicate that inhibition of photosynthesis is probably not an important site of action for SC-0224.

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V. SUMMARY AND CONCLUSIONS

The primary objective of this research was to compare the modes of action of the herbicides SC-0224 and glyphosate. This study has provided further evidence supporting previous findings, that there are both similarities and differences in the herbicidal action of the two compounds.

This research on inflated duckweed growth demonstrated that there are striking differences in the action of SC-0224 and glyphosate. The results indicated that SC-0224 is much more phytotoxic than glyphosate on this species and that this phytotoxicity could not be prevented by treatments involving supplemental aromatic amino acids. The data also indicate that trimethylsulfonium iodide (TMS-I) has phytotoxic effects similar to that of SC-0224 on inflated duckweed. This finding suggests that differences in the action of SC-0224 and glyphosate may be due to the phytotoxic effects of TMS.

The results of the amino acid and protein studies also indicated that differences in the action of the two herbicides may be due to the independent action of the TMS ion. These studies showed that SC-0224 and TMS-I caused similar changes in amino acid concentrations, soluble protein levels, and protein synthesis. However, the changes on these systems caused by glyphosate were significantly different from those caused by SC-0224 and TMS-I. Although the specific cause of the rise in free amino acid pool levels was not

determined, is is concluded that these changes may be due to the action of the TMS ion.

The data indicate that the rises in amino acid concentrations are probably not due to decreased protein synthesis alone since the per gram fresh weight data demonstrated no significant differences for the protein synthesis study; whereas, the amino acid pools were greatly different on a per gram fresh weight basis. Also, if the increased amino acid concentrations were due to decreased protein synthesis or increased protein hydrolysis, one might expect to see increased concentrations of more of the amino acids. However, interconversions of amino acids may occur which would explain some of the differences. Because of the inhibition of growth effected by SC-0224 and TMS-I, one may expect that respiration is reduced in treated plants. Therefore, decreased use of amino acids as carbon sources is a likely cause of the increased amino acid pools as discussed in chapter III. Since there is no clear link between all of the amino acid concentrations affected, it is likely that TMS affects the amino acids pools indirectly. These altered amino acid pools may thus result from any or all of the factors listed in chapter III.

An aquatic species, such as inflated duckweed, may respond very differently from terrestrial species to glyphosate, SC-0224, and/or TMS for a number of reasons. In this study the inflated duckweed plants were in continuous contact with the treatment solutions which removes the need for rapid penetration into the plant. The herbicide may also be taken up by the roots of this aquatic species which would not be possible with a foliar application of a

terrestrial species. Since inflated duckweed plants are small, the need for translocation of the herbicide is reduced. The action of SC-0224 and glyphosate should be much more similar on terrestrial species than on inflated duckweed if SC-0224 ionizes in solution and the TMS ion penetrates and/or translocates poorly in terrestrial species.

The data of the EPSP-synthase studies give a strong indication that SC-0224 does in fact ionize in solution. If TMS remained bonded to N-(phosphonomethyl)glycine (PMG), the structural alteration would probably change the action of PMG on EPSP-synthase activity. This study indicates that SC-0224 and PMG are equally potent inhibitors of EPSP-synthase and that TMS-I has no effect on this enzyme. If, as suggested above, the TMS ion does not penetrate and/or translocate to active sites in terrestrial species, SC-0224 and glyphosate may have identical sites of action in such species. However, additional studies are needed to determine the penetration and translocation properties of TMS. Comparisons of these chemicals on a wide range of species are also needed since differences between TMS, SC-0224, and glyphosate may be species dependent.

This research has provided new data on the action of TMS; however, the primary site of action is probably not any of the systems studied here since TMS does not inhibit EPSP-synthase or the Hill reaction and TMS does not directly alter protein synthesis or soluble protein levels. The increased amino acid pools are probably due to a combination of factors which may be secondary to the primary site of TMS action. The data have demonstrated an effect of TMS on

water content in inflated duckweed. This was a statistically significant but small difference in water content and may thus indicate that TMS affects membrane integrity; however, additional research is needed to confirm this hypothesis.

In summary, this research has shown that the action of SC-0224 probably results from the individual action of TMS and PMG, that SC-0224 and PMG both inhibit EPSP-synthase activity, and that none of the chemical treatments inhibits the Hill reaction. These studies have determined some of the biological actions of TMS although the site of action was not determined. From a practical standpoint, the herbicidal action of SC-0224 and glyphosate may not be greatly different in the terrestrial species on which they are most commonly used. However, rates and routes of foliar penetration and translocation — therefore rapidity in manifesting phytotoxic action — may be reasonably expected to differ between the two compounds and among plant species sprayed due to the influence of the different cationic constituents (TMS and isopropylamine) present in the two herbicide products. In any case, additional research is needed to determine more precisely the action of SC-0224 in other species and to determine the site and/or mechanism of action of TMS.

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