1981

Interactions of Mefluidide and Bentazon on Red Rice (Oryza Sativa L.).

Sudabathula Rajaramamohana Rao

Louisiana State University and Agricultural & Mechanical College

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INTERACTIONS OF MEFLUIDIDE AND BENTAZON ON RED RICE (ORYZA SATIVA L.)

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Plant Pathology and Crop Physiology

by

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December 1981
ACKNOWLEDGMENTS

The author wishes to express his gratitude to the members of his dissertation committee, Dr. J. B. Baker, Dr. K. L. Koonce, Dr. E. P. Dunigan, and Dr. L. M. Kitchen for their constructive criticism of the manuscript.

The author is grateful to Dr. T. R. Harger, committee chairman, for his guidance, assistance, and patience during the course of his research.

The educational opportunities and the research assistantship provided by the Department of Plant Pathology and Crop Physiology was greatly appreciated.

The author gratefully acknowledges the technical assistance of Ms. L. Lyons, Ms. P. Johnsey, Ms. J. Rao, Mr. T. Terrell, Mr. R. Shah, Mr. R. Arimilli, Mr. D. Goyer, Ms. J. Thompson, Mr. R. Martin, Mr. A. Malcolm, Mr. M. Donnelly, Ms. J. Evans, and Dr. N. Fischer.

Special thanks are extended to fellow graduate students and research associates, Paul Nester, Jim Shrefler, Robert Prince, Scotty Crowder, Eddie Millhollon, Jeff Yoder, Vernon Langston, Ralph Helms, Gerald Dill, Gerard DiMarco, and Mafizur Rahman for their understanding and encouragement.

The author is grateful to his parents and relatives for not bothering him with family matters during his years as a graduate student.

Finally, the author wishes to thank his wife, Junette and daughter, Jamuna for their patience and understanding during the course of his graduate studies.
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ABSTRACT

The responses of soybeans [Glycine max (L.) Merr. 'Bragg'] and red rice (Oryza sativa L. 'Strawhulled') to postemergence applications of mefluidide [N-[2,4-dimethyl-5-[[trifluoromethyl)sulfonyl]amino]phenyl] acetamide] and bentazon [3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] alone and in combinations were evaluated in greenhouse studies. Soybeans and red rice were tolerant to bentazon, but their heights were reduced by mefluidide. When these two herbicides were combined, soybeans were not injured at any tested rates, but a synergistic interaction occurred that killed red rice.

The changes in degradation, uptake, translocation, metabolism, and loss of these two herbicides due to combinations with each other were studied in order to establish the basis for synergistic interactions of this herbicide combination on red rice. The combinations of these herbicides resulted in decreased degradation and uptake of both herbicides and decreased loss of only mefluidide as compared to either used alone. The translocation of both herbicides was primarily acropetal with limited basipetal movement. The translocation of $^{14}$C from mefluidide from the treated-middle leaf was reduced by bentazon sprayed over-the-top, however, the entire plant showed necrosis. Mefluidide, sprayed over-the-top, increased the translocation of $^{14}$C from bentazon, but necrotic symptoms appeared only on the bentazon-treated middle leaf.

There were no significant changes in the metabolism of mefluidide due to addition of bentazon. Mefluidide reduced the rate of metabolism and conjugation of bentazon by red rice, and consequently high levels of free bentazon were maintained within the plant. Application of mefluidide 32 h following that of bentazon also resulted in a synergistic
response by red rice. Although all the bentazon absorbed by red rice prior to delayed application of mefluidide was conjugated within 32 h, the mefluidide treatment prevented further conjugation of bentazon which continued to enter the plant. The identified metabolites of these two herbicides in red rice were not toxic to the plant.

The inhibition of bentazon detoxification by mefluidide in red rice appears to result in the apparent synergistic interactions between the herbicides. Free bentazon remaining in the plant probably caused necrosis and death of red rice.
INTRODUCTION

Red rice is a weedy variety of cultivated rice (Oryza sativa L.) and is considered to be the worst weed in rice fields of southwest Louisiana (99) and parts of the southern rice belt of the United States (245). Red rice presents a challenging control problem in the rice crop (21,129,245) because of the physiological (131,235) and morphological (131) similarities of the two varieties. Red rice grows taller than cultivated rice (245), tillers profusely (131), and contributes to lodging (245). This causes reduced grain yields (228,245). Red rice lowers the grade of milled rice (131,228) because of its objectionable dark-brown to red bran layer (21,131,228,235). Removal of this dark pericarp requires extra milling which causes excessive breakage and damage to the cultivated rice grains (131,228). Because of its competition with cultivated rice and infestation in harvested rice, red rice causes an estimated loss of $50 million each year in the southern rice belt (245). Red rice shatters readily before harvest (131,235,245), thus establishing a population of dormant seed in the soil (115,235) that can remain viable for 7 to 12 yr (115). The seed remains alive much longer under irrigated conditions as is usually the case with rice fields as compared to unirrigated conditions (115).

Fontenot (99) discussed the possibilities of inhabiting wild ducks during fall and winter months to consume all of the red rice in the top zone of the soil. This method, however, is impracticable as it requires several deep ploughings to bring the red rice seed up to the soil surface and standing water throughout the season to make the fields attractive to ducks. Soybeans [Glycine max (L.) Merr.] have been recommended in a two-year rotation followed by rice crop the third year.

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as an effective means of reducing the red rice population in the soil between rice crops (244, 245). Five percent or less escapees of red rice do not affect soybean production adversely, but can produce enough seed to restore original soil population levels (115). Therefore, complete control of red rice is more critical than with other weeds in soybeans.

Preemergence herbicides are not effective (245) as red rice can emerge from deeper layers of soil and herbicides may not be present in zones where red rice germination occurs (129). Several researchers recommended preplant incorporation of double the normal rates of alachlor [2-chloro-2',6-diethyl-N-(methoxymethyl)acetanilide] (6, 245), trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) (7, 245), proflurain [N-(cyclopropylmethyl)-a,a,a-trifluoro-2,6-dinitro-N-propyl-p-toluidine] (245), and vernolate (5-propyl dipropylthiocarbamate) (244), or normal rates of application of metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] (122), trifluralin plus metribuzin [4-amino-6-tert-butyl-3-(methylthio)-atrizin-5(4H)-one] (245), and proflurain plus metribuzin (245) to reduce the emergence of red rice. The only effective means of controlling those plants that escape preplant incorporated herbicides is by cultivation (99) or directed postemergence applications of chloroxuron [3-[p-(p-chlorophenoxy)phenyl]-1,1-dimethylurea] (244) or paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) (245). In the major rice production areas of southwest Louisiana, most farmers prefer to grow soybeans in a solid seeded culture which eliminates both cultivation and directed sprays. Diclofop [2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid] was reported to give acceptable control of red rice in soybeans as an early postemergence treatment (84, 135). The results were incon-
sistent, however, and the control was unacceptable in the field experiments at various locations in Louisiana\textsuperscript{1} and Arkansas (174). Mefluidide \(N-[2,4\text{-dimethyl-5-}[[\text{(trifluoromethyl)sulfonyl}]]\text{amino}]\text{phenyl}]\text{acetamide}\) and bentazon \(3\text{-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide}\) applied in combination as an early postemergence treatment to soybeans controlled red rice (16,83,85,86,101,123,269) and other problem weeds (16,83,86,104,123,182,237,238,269) more effectively than either herbicide applied alone (16,85,104,123,182,269) indicating an additive or synergistic effect. Slight crop injury (44,83,101,124,182,290) and lodging of soybeans (124) was noted with mefluidide treatments of 0.5 kg/ha. Bentazon, however, was reported to cause minimal soybean injury at rates as high as 1 kg/ha (23,124,237,277,287).

The objectives of these studies were to determine a) the tolerance of soybeans to mefluidide and bentazon applied alone and in combination, b) the response of red rice to different rates of mefluidide and bentazon applied alone and in combination with and without a surfactant, c) the response of red rice to sequential applications of mefluidide and bentazon, and d) the changes in degradation, uptake, translocation, metabolism, and loss of mefluidide and bentazon by red rice due to combination with each other in order to elucidate the basis for synergism in red rice.

\textsuperscript{1}Rao, S.R., unpublished data.
LITERATURE REVIEW

Mefluidide is a postemergence soybean-herbicide manufactured by the 3M Company. The morphological responses of several crops and weeds to this herbicide have been studied thoroughly both in the greenhouse and in the fields. However, very limited information is available on the physiological or biochemical activity of mefluidide other than uptake and translocation by selected plant species. Therefore, the literature on mefluidide and selected compounds that have structural and functional similarities with mefluidide will be reviewed in Sections A to G.

Bentazon is a selective postemergence herbicide developed in the late 1960's by BASF Wyandotte Corporation, the American subsidiary of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany (3). The activity, selectivity, uptake, translocation, and metabolism of bentazon has been studied extensively in several crops and weeds. Since it is an unclassified herbicide, the review presented in Sections H to 0 will be limited only to bentazon.

The interactions between herbicides, herbicides and antidotes, and herbicides and insecticides will also be reviewed here in order to establish a background on how chemicals may interact to cause a synergistic or antagonistic response in plants.

A. Physical and Chemical Properties of Mefluidide

Mefluidide is an odorless, white, crystalline solid with a molecular weight of 310.3. The compound is weakly acidic (pKₐ 4.6), forms salts (44), stable to heat and moisture (258) with a melting point of 183-185 °C (44). Mefluidide is relatively resistant to decomposition by ultraviolet radiation (270), but susceptible to photodecomposition.

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in solution (258,270). Mefluidide is not very soluble in water (180 mg/L) and benzene (300 mg/L), but is readily soluble in acetone (3.5 x 10^5 mg/L), methanol (3.1 x 10^5 mg/L), acetonitrile (6.4 x 10^4 mg/L), and ethyl acetate (5 x 10^4 mg/L) at 22-23 C (93,108). Mefluidide is nonvolatile with a vapor pressure of <10^-4 mm Hg at 25 C. Vistar® is the trade name (259) for the water-soluble diethanolamine salt of mefluidide (240 g/L) (258,270). The chemical structure of mefluidide is depicted in Figure 1.

B. Activity and Selectivity of Mefluidide

Mefluidide belongs to the amide chemical class of herbicides (general formula, Figure 2). The amide herbicides comprise a diverse group of chemicals with various substitutions (13). Most amides are used for preemergence weed control, i.e., alachlor, butam [2,2-dimethyl-N-(1-methylethyl)-N-(phenylmethyl)propanamide], CDAA (N,N-diallyl-2-chloroacetamide), diethyl [N-(chloroacetyl)-N-(2,6-diethylphenyl)glycine], diphenamid (N,N-dimethyl-2,2-diphenylacetamide), metolachlor, napropamide [2-(α-naphthoxy)-N,N-diethylpropionamide], pronamide [3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide], and propachlor (2-chloro-N-isopropylacetanilide) (structural formulae, Figure 2). Propanil (3',4'-dichloropropionanilide), solan [N-(3-chloro-4-methylphenyl)-2-methylpentanamide] (structural formulae, Figure 2), and mefluidide, however, are applied postemergence to the weeds to be controlled.

Mefluidide has shown promise for potential uses such as weed control and suppression, grass growth regulation and seedhead suppression, tree and ornamental plant growth regulation, sucrose enhancement in sugarcane (Saccharum officinarum L.), and forage crop quality improvement (258).
Figure 1. Structural formula of mefluidide \( \text{N-}[2,4\text{-dimethyl-5-} \[
\text{[trifluoromethyl]sulfonyl}\text{amino}]\text{phenyl}]\text{acetamide} \).
Figure 2. General and structural formulae of various amide herbicides.
I. Weed Control

The 3M Company obtained an experimental use permit in 1978 (106, 259) for the use of mefluidide as a postemergence herbicide for the control of seedling and rhizome johnsongrass \textit{(Sorghum halepense (L.) Pers.)}, shattercane \textit{(Sorghum bicolor (L.) Moench)}, volunteer sorghum \textit{(Sorghum bicolor (L.) Moench)}, and volunteer corn \textit{(Zea mays L.)} in soybeans. Under this permit, mefluidide was recommended at 0.28 kg/ha with a non-ionic surfactant (0.25 to 0.5%, v/v) in water at a rate of 93 to 274 L/ha as an over-the-top or directed spray to soybeans. The first application of this herbicide was recommended when soybeans are actively growing and only after the second trifoliolate leaf has expanded. For the best results, the label specified that mefluidide should be sprayed when seedling or rhizome johnsongrass is less than 38 cm or when shattercane, volunteer corn, and volunteer sorghum are 38 to 51 cm tall. An optional second application of mefluidide at 0.14 kg/ha is also recommended at 3 to 4 weeks after the first application if rapid regrowth of the weeds occurred and the soybean canopy is not shading the weed foliage (259).

a. Johnsongrass Control

The control of johnsongrass with mefluidide has received much attention recently because johnsongrass is one of the hard-to-kill weeds, and mefluidide is known to have growth regulating activity on this weed. Massey (172) tested mefluidide for the control of johnsongrass in several crops and found that both seedling- and rhizome-johnsongrass were susceptible to this herbicide. Bushong et al. (44) obtained 100% initial control of young johnsongrass seedlings at rates of 0.28 to 1.12 kg/ha in soybeans, but observed a slight regrowth when the plants were rerated 49 days later bringing the control down to 93.
to 98%. Gates et al. (107) obtained 81, 87, and 93% control of 15 to 38 cm-tall johnsongrass when mefluidide was applied at 0.28, 0.56, and 1.12 kg/ha, respectively. These results are further substantiated by the findings of Hargroder et al. (123) who also obtained a consistent control of johnsongrass up to 38 cm tall when mefluidide was applied either as an over-the-top or directed spray in soybeans. The control was 81 and 87% at 0.28 and 0.56 kg/ha, respectively.

McWhorter and Barrentine (182) tested mefluidide at various rates and frequencies of application for johnsongrass control in soybeans. They obtained 80 to 85% control of 15 to 28 cm-tall johnsongrass with mefluidide applied as an over-the-top spray with a single application at 0.9 kg/ha or two applications each at 0.44 kg/ha. Directed sprays at 0.22 to 0.67 kg/ha were found to be more effective on johnsongrass than similar treatments applied overtop. Johnsongrass control was improved when plots were pretreated with trifluralin at 0.56 kg/ha as a preplant incorporated application.

Several workers reported only growth suppression of johnsongrass without much effect on its population due to postemergence applications of mefluidide. Glenn and Rieck (110) reported that mefluidide at 0.28, 0.56, and 1.12 kg/ha gave 80 to 95% growth suppression of 45 cm-tall johnsongrass in soybean fields. They attributed this growth suppression to translocation of mefluidide to apical meristems resulting in prevention of floral development. Similar results were also reported by Rogers et al. (238) who obtained 60 to 90% growth suppression of johnsongrass with 0.28 to 0.56 kg/ha of mefluidide and less suppression at 0.14 kg/ha. Connell and Jeffery (60) investigated johnsongrass control in no-till soybeans using mefluidide as a preplant foliar
treatment. They found that mefluidide at 0.56 kg/ha did not reduce the population of 102 cm-tall johnsongrass, but did reduce the height by 50%. They concluded that preplant foliar application of mefluidide could suppress johnsongrass, but this would not be an adequate treatment for no-till soybeans.

Johnsongrass control was reported to become more erratic and less effective as plants became mature (61,107,123,229,257). Mefluidide applied mid- to late-season prevented seedhead formation (107), but applications at boot stage were found to be only marginally effective (257).

The addition of nonionic surfactants to mefluidide was found to increase the consistency of johnsongrass control (106,107,123) and only the lower rates of the herbicide were required for effective control (124). McWhorter and Barrentine (182) reported a 76 to 90% control of johnsongrass due to addition of 0.5% (v/v) nonoxynol [a-(p-nonyl-phenyl)-ω-hydroxypropoxy (oxyethylene)] to 0.44 or 0.67 kg/ha mefluidide applied either directed or over-the-top. Without a surfactant, mefluidide was required at 0.9 kg/ha for the same degree of control.

In most reported cases, the regrowth of treated johnsongrass was suppressed for 3 to 6 weeks following mefluidide application (44,107). This regrowth was reported to be prevented with either split applications of mefluidide at low rates starting on young johnsongrass (109, 256) or with an optional second application at 0.14 to 0.28 kg/ha rate (123). The control level of johnsongrass seems to improve when a dense canopy develops from a good stand of actively growing soybeans (106,107).

The mefluidide-treated johnsongrass was reported to exhibit terminal kill, twisting, stunting, and withering to near soil level (107) thus allowing soybeans to grow with minimum competition (106).
b. Control of other weeds

Many researchers have obtained effective control of various other weed species in several crops tested. Good to excellent control of hemp sesbania \textit{[Sesbania exaltata (Raf. Cory)]} (104,182,237,238), common cocklebur \textit{(Xanthium pensylvanicum Wallr.)} (182,237,238,290), volunteer corn, volunteer grain sorghum (123), redroot pigweed \textit{(Amaranthus retroflexus L.)} (237,242), wild mustard \textit{[Brassica kaber (DC.) L.C. Wheeler var. pinnatifida (Stokes) L.C. Wheeler]} (242) and several grasses (237) were reported with mefluidide applied postemergence at used rates of 0.28 to 1.12 kg/ha. Addition of a surfactant, in general, was found to improve upon weed control.

Other reports indicated postemergence activity of mefluidide on hemp sesbania, common cocklebur, morningglories \textit{(Ipomoea spp.)} (44,172), common sunflower \textit{(Helianthus annuus L.)}, mustards \textit{(Brassica spp.)}, sowthistles \textit{(Sonchus spp.)}, wild oats \textit{(Avena fatua L.)}, giant foxtail \textit{(Setaria faberi Herrm.)}, volunteer sorghum, and a number of seedling grasses (172) in tolerant crops such as beans \textit{(Phaseolus spp.)}, sugarbeets \textit{[Beta vulgaris (L.) Beauv.]}, safflower \textit{(Carthamus tinctorius L.)} (172), and soybeans (44).

Mefluidide at rates of 0.28 to 1.12 kg/ha was also reported to be ineffective in controlling many common weed species including redroot pigweed (124,238), fall panicum \textit{(Panicum dichotomiflorum Michx.)}, common cocklebur (124), prickly sida \textit{(Sida spinosa L.)} (51,61,237,238), velvetleaf \textit{(Abutilon theophrasti Medic.)} (51), yellow nutsedge \textit{(Cyperus esculentus L.)} (61), spurred anoda \textit{[Anoda cristata (L.) Schlecht.]} (51), Venice mallow \textit{(Hibiscus trionum L.)}, morningglories \textit{(Ipomoea spp.)} (51,124,237,238,263), yellow foxtail \textit{[Setaria lutescens (Weigel) Hubb.]},

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barnyardgrass \(Echinochloa \text{ crus-galli} \) (L.) Beauv., wild oats, common lambsquarters \(Chenopodium \text{ album} \) L.), and kochia \(Kochia \text{ scoparia} \) (L.) Schrad.]. The conflicting results on the control of certain above mentioned weed species could be due to variations in the time and rate of mefluidide application, presence or absence of a surfactant, and the observation date.

In common cocklebur and hemp sesbania, only a terminal kill of the growing points was initially observed with regrowth occurring later (104,124). Other symptoms observed on treated plants were deformed apical buds, complete kill of apical buds and increase in secondary branching as in peaches \(Prunus \text{ persica} \) (L.) Batsch] (103), delay in flowering and pollination, and increase in tillering and reduction of seedhead production of grasses (242).

c. Herbicide Mixtures

The combinations of mefluidide with other herbicides were reported to broaden the spectrum of weeds controlled, result in acceptable control of taller weeds, and cause additive or synergistic control of certain weed species (238,269).

Additive or synergistic control of taller hemp sesbania (16,104, 123,182,269) and improved or synergistic control of morningglories \(Ipomoea \) spp.) (123,238), common cocklebur (123,182), taller redroot pigweed (238), and prickly sida (237) were reported with the combinations of mefluidide and bentazon as compared to either herbicide alone. These results suggest that for effective control of these weed species, the rates of mefluidide could be lowered when combined with other herbicides (104,238).

Rogers et al. (238) noted that when mefluidide and bentazon were
used in combination, increasing the rates of bentazon from 0.56 to 1.12 kg/ha gave 20% better control of redroot pigweed and morning-glories (*Ipomoea* spp.) whereas there was no effect of increasing the rates of mefluidide when these herbicides were in combination.

Mefluidide was also tested in combination with several other herbicides. Connell and Jeffery (60) reported a synergistic height reduction of johnsongrass where mefluidide was applied following the application of glyphosate [N-(phosphonomethyl)glycine]. The combination of mefluidide and chloroxuron were reported to result in additive or synergistic kill to hemp sesbania (104,123), prickly sida (237), morning-glories (*Ipomoea* spp.), and common cocklebur (123). McWhorter and Barrentine (182) obtained better control of hemp sesbania and common cocklebur where mefluidide was combined with dinoseb (2-sec-butyl-4,6-dinitrophenol) or dinoseb plus naptalam (N-1-naphthylphthalamic acid) than where they were applied alone.

In general, the combinations of mefluidide with fluometuron [1,1-dimethyl-3-(α,α,α-trifluoro-m-tolyl)urea] or methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] were additive for the control of prickly sida (51,61), Venice mallow, velvetleaf, four *Ipomoea* spp., and spurred anoda (51), whereas the combinations with MSMA (monosodium methanearsonate) were synergistic on four *Ipomoea* spp., and additive on Malvaceae spp. and prickly sida (61).

Contrary to the previous reports, Harrison et al. (124) did not observe any advantage either in the selectivity of soybeans or the control of 5 to 18 cm-tall johnsongrass, redroot pigweed, morningglories (*Ipomoea* spp.), or common cocklebur due to combinations of mefluidide with chloroxuron or bentazon. Complete kill of these young weeds was
probably obtained when these herbicides were applied alone, and consequently the advantages of combination could not be estimated.

II. Crop Injury

Mefluidide provides selective weed control in soybeans, cotton (*Gossypium hirsutum* L.) (51,103), peanuts (*Arachis hypogaea* L.) (264, 286), and other field crops. Increasing the rates or addition of a surfactant, however, can decrease crop selectivity resulting in lower yields. The type of foliar injury due to mefluidide included height reduction (182,237), lodging (124), and crinkling and mottling of leaves (110,182).

Several workers reported soybean injury due to mefluidide at 0.56 kg/ha or higher (44,83,109,110,124,237,290), but in some cases, soybeans were found to recover from injury at later stages of growth (44,269). Soybean yield reduction, however, was reported with mefluidide at 1.12 kg/ha applied to 2- to 5-trifoliolate soybeans (109,110,290).

McWhorter and Barrentine (182) evaluated soybean injury at various application rates of mefluidide. They noted 0% injury for one application of mefluidide at 0.44 kg/ha to 32% for two applications at 0.9 kg/ha. Soybean injury was increased by addition of a surfactant. The 32% level of soybean injury caused by two applications of 0.9 kg/ha mefluidide resulted in significant reduction of soybean yields as compared to plots treated with two applications each at 0.44 kg/ha or a single application at 0.9 kg/ha. A decrease in soybean injury due to mefluidide, however, was reported when mefluidide was combined with bentazon as a tank mix indicating an antagonistic activity on soybeans (30).

Increase in soybean yields were also reported (107,110,237) due to
control of johnsongrass and other weeds with mefluidide at rates (0.28 to 0.56 kg/ha) that were not injurious to soybeans. The average increase in soybean yields were 336 kg/ha (106,107).

Crawford and Blackmon (61) did not observe any injury on 15 to 38 cm-tall cotton with 0.56 kg/ha mefluidide, whereas Chandler (51) reported reduced cotton yields (6 to 14 leaf-stage) at 0.57 kg/ha rate. Schweizer and Eshel (242) reported a desirable suppression of foliar growth of sugarbeet at all stages of growth even at low rates (0.34 kg/ha) of mefluidide application.

III. Growth Regulation

Mefluidide, at low rates of application, has a growth regulating activity although herbicidal activity is more predominant at high rates. The 3M Company has registered mefluidide under the trade name, "Embark" as a plant growth regulator in turf crops (258). Gates and Pauly (105) discussed potential uses of mefluidide for reducing vegetative growth and seedhead production of cool and warm season grasses. Many researchers, since then, have reported mefluidide at low rates to: a) retard growth of grasses (44,56,103,172,186,202,217,241,249,271,272,273,274,285) without reducing root growth in turf (Poa pratensis L., Agrostis tenuis Sibth, Festuca rubra L.) (103,202); b) suppress seedhead production (44,56,103,138,172,202,242,249,285); c) enhance the color of turfgrass (Festuca rubra L.) (103,249); d) decrease the mowing frequency of grasses (44,186,249,272); e) delay flowering and pollination (242); f) decrease vine growth of peanuts (264,286); g) decrease the formation of suckers in tobacco (Nicotiana tabacum L.) (44); h) suppress vegetative growth of trees and woody ornamentals (44,103,172); and j) suppresses apical meristem growth and increase secondary branching of peaches (103).
Chappell et al. (56) tested mefluidide (0.28 to 1.12 kg/ha) with a surfactant at three locations on red fescue (Festuca rubra L.) and tall fescue (Festuca arundinacea Schreb.) on the rights-of-way of highways to determine the extent of inhibition of vegetative growth and seedhead production. With increasing rates of mefluidide, there was a more effective suppression of both grasses, but foliar burn was noted at the highest used rate of 1.12 kg/ha. In general, the growth suppression was observed for 3 to 8 weeks following a single (44,56,138,186,202,217,242,249,271,272,273,274) or split application (285) of 0.28 to 0.56 kg/ha of mefluidide on most of the grass species tested (44,186).

Watschke et al. (274) and Nielson and Wakefield (202) observed a significant reduction in the growth of turfgrasses (Poa pratensis L., Agrostis tenuis Sibth, Festuca rubra L.) treated with mefluidide often followed by a stimulation in the growth of these grasses later in the season. This growth stimulation, later in the season, could be prevented by reapplication of mefluidide (274).

The turf and lawn grasses that were found to be retarded by the applications of mefluidide include red fescue (56,138,202,217,249), Kentucky bluegrass (Poa pratensis L.) (138,202,217,241,271,273,274), tall fescue (108,111,272), colonial bentgrass (Agrostis tenuis Sibth) (138,202), perennial ryegrass (Lolium perenne L.) (138) and bermuda-grass [Cynodon dactylon (L.) Pers.] (44,186,285).

IV. Forage Quality

Many turfgrasses are also common forage crops. The growth regulation and the color changes caused due to application of mefluidide might reflect the forage quality of these turfgrasses.

Watschke (271) reported a significant accumulation of total non-
structural carbohydrates (TNC) in Kentucky bluegrass treated with mefluidide after the initial discoloration subsided. Nonstructural carbohydrates are immediate products of photosynthesis which would tend to accumulate in treated turf due to growth suppression. This accumulated TNC could then provide energy for regrowth once the chemical stress has dissipated (271). The rapid utilization of the conserved TNC later in the season might explain the post-inhibition growth stimulation (202,271,272,274) and enhanced color of turf grasses (138,271,272) that has been reported. Mefluidide-treated tall fescue was reported to contain increased levels of sugars due to inhibition of floral development, higher levels of crude protein due to stimulation of protein synthesis, and decreased cellulose content due to inhibition of reproductive growth and metabolic changes (108). These metabolic changes in tall fescue may offer the potential to improve forage quality without affecting dry matter yields by the application of mefluidide (108).

Briskovich (39) also observed that low concentrations of mefluidide tended to increase crude protein content of red- and tall fescue while high concentrations (1.12 and 2.24 kg/ha) caused leaf discoloration and reduced protein content.

V. Sugar Crops

Chemical ripening of sugar cane with synthetic growth regulators such as mefluidide has been of interest to sugarcane industry. There are several reports on early ripening of sugarcane (44,57,239,261), and increased total sugar (44,172) and recoverable sugar (103) contents due to mefluidide application. This chemical treatment would ultimately allow early sugarcane harvest as well as increase the yields of sugarcane. Schweizer and Eshel (242), however, reported a decrease
in sucrose content of sugarbeet treated with mefluidide at high rates (0.68 and 1.02 kg/ha) and subsequently subjected to stress conditions.

C. Influence of Environmental Factors on the Activity of Mefluidide

The literature concerning the influence of environmental factors on the activity of mefluidide is very limited. The 3M Company (259), under its experimental use permit, has recommended that mefluidide not be used when soybeans are under moisture stress, damaged by insects or diseases, or suffering from injury caused by other pesticides. Atwell and Viar (16) reported leaf crinkling and bronzing of mefluidide-treated soybeans due to high temperature, high relative humidity (RH), and low soil moisture. Soybeans, however, were also reported to recover from this injury later in the season.

Glenn and Rieck (111) studied the influence of temperature on shoot and root mass of mefluidide-treated tall fescue. Tall fescue was treated with 1.12 kg/ha mefluidide, and then plants were subjected to 29 C. Two weeks after the treatment, shoot mass was found to be unaffected, but root mass was decreased by 53.6%. When some of the mefluidide-treated plants were subjected to 29 C for two weeks followed by 1 C for 5 days and then 29 C for 1 week, both shoot and root mass were decreased by 27.2 and 23.9%, respectively. They concluded that the stress caused by low temperature resulted in significant reduction of shoot weight of mefluidide-treated tall fescue. Swisher and Kapusta (257) also observed a reduction in control of johnsongrass due to dry weather.

D. Biochemical and Physiological Responses to Mefluidide

Visual observations of the plants treated with mefluidide reveal suppression of growth and development accompanied by callus formation.
near the base of the plant and lack of floral development probably through interference with the normal functioning of meristematic tissues of the plant (108).

Glenn and Rieck (112) reported a stimulation in the elongation of coleoptiles of corn at very low concentrations of (0.001 mM) mefluidide similar to that caused by indole acetic acid (IAA) (108). A concentration of 0.1 mM, however, was found to be inhibitory and this inhibition was reported to be reversed by the application of IAA.

Briscovitch (39) examined the influence of several plant growth regulators on mefluidide-produced growth responses and concluded that IAA might be involved in the mefluidide response although he did not find mefluidide to be inhibitory to \(^{14}\)C-IAA transport. He suggested that the major mode of action of mefluidide in tall fescue could be disruption of IAA metabolism.

Glenn (108) and Glenn et al. (114), on the other hand, demonstrated the influence of mefluidide on the basipetal transport of \(^{14}\)C-IAA. Mefluidide at \(10^{-3}\) M was found to inhibit \(^{14}\)C-IAA transport by 81.5%, while a lower concentration (\(10^{-4}\) M) was found to increase this transport by 246.5%. They concluded that mefluidide at low concentrations stimulates the movement of IAA to the areas to which auxins are applied.

Glenn (108) also used several compounds (Figure 3) structurally related to mefluidide in order to study the influence of various functional groups of mefluidide on IAA transport. MBR-22441 (6-acetamide-m-xylene), MBR-15733 (6-acetamide-4-amino-m-xylene, and MBR-12130 (4,6-diamino-m-xylene) at \(10^{-4}\) M were reported to exhibit no activity on IAA transport. This indicates that the acidic trifluoromethyl sulfonyl amino side chain of mefluidide is required to affect IAA transport and,
Figure 3. Chemical structures of mefluidide and mefluidide-related compounds.
therefore, is probably required for auxin activity of mefluidide. Koepfli et al. (148) reported that an acidic side chain is required for a molecule to exhibit auxin activity. MBR-15733 and MBR-12130 are the only known metabolites of mefluidide in plant (108), neither exhibiting auxin activity. These observations suggest that the auxin activity of mefluidide is probably exerted by the parent molecule rather than one of its metabolites. MBR-4410 (4-trifluoromethylsulfonyl-amino-o-xylene) at 10^{-4} M was found to increase the IAA transport by only 97%. Removal of acetamide side chain probably reduces the auxin activity of mefluidide. Koepfli et al. (148) reported that certain spatial relationship between the acidic side chain and the unsaturated ring may be required for auxin activity of a molecule. Muir et al. (195) found that the lipophilic and electronic factors of the substituents of phenylacetic acids are important in determining the auxin activity of mono-substituted phenylacetic acids. If this is the case, removal of acetamide side chain might alter either the spatial relationship or lipophilic nature and electron density of mefluidide which may be important factors in its activity as an auxin. MBR-7200 (3-trifluoromethylsulfonylamino-o-acetotoluidide) was found to increase IAA transport by 255%, equal to that produced by mefluidide at the same concentration of 10^{-4} M. Fluoridamid (N-[3-[(1,1,1-trifluoromethylsulfonyl)amino]-4-methylphenyl]acetamide) at 10^{-4} M was reported to decrease IAA transport by 39% whereas a lower concentration (10^{-5} M) was found to increase the transport by 61%. Fluoridamid appears to have a greater activity on IAA transport than mefluidide because only lower concentrations of fluoridamid are required to decrease IAA transport. Katekar (143) states that the effect of substituents on molecules is to increase activity if they have available electrons to bind, but to
decrease activity if they do not. He supports the theory that only aromatic rings and halogens give rise to high activity while other substituents including methyl groups have only comparatively weak effects. He suggests that only the compounds that have appropriate atoms covering postulated critical areas would have auxin activity, while others that cover only portion of the critical areas would have only weak activity. The observed results on the activity of MBR-7200 and fluoridamid would not be inconsistent with the hypothesis of Katekar due to nature of the substituent and type of substitution on these two compounds as compared to mefluidide.

Truelove et al. (267) reported that mefluidide-induced growth reduction of corn could not be reversed by single or repeated application of GA3 (gibberellins). However, mefluidide was able to prevent those GA3-promoted changes which result in cell enlargement. Fluoridamid was also reported to inhibit the GA3 biosynthesis pathway of castor bean (Ricinus communis L.) enzyme preparations (247). Based on these observation, Truelove (266) suggested that mefluidide may alter nucleic acid metabolism which could not be overridden by GA3 application in corn. Similar conclusions were also reached by Briscovich (39) working with interactions of mefluidide and GA3 on tall fescue.

These observations suggest that growth retardation effects of mefluidide are probably due to its auxin-like activity rather than through the inhibition of GA3 biosynthesis. This auxin-like activity might have partly contributed to the observed twisting, distortion, and regulation of growth that is characteristic of mefluidide-treated plants. It should, however, be noted that herbicidal activity of mefluidide is more predominant at high rates of applications which ultimately causes the death of the plant.
Glenn et al. (114) and Glenn (108) reported an increased incorporation of $^{14}$C-labelled precursors into protein, RNA, and DNA by 37.3, 43.4, and 47.7%, respectively, with mefluidide at $10^{-6}$ M concentration. Based on their results, these authors attributed this increase in the synthesis of these three components to auxin activity. They suggested that mefluidide may stimulate activity or production of auxin, or may act as an auxin and that this auxin activity probably enhances cell growth and RNA-directed protein synthesis in tall fescue.

Truelove et al. (267) also observed an increase in the incorporation of $^{14}$C-leucine into protein due to mefluidide at a low concentration of $2.9 \times 10^{-5}$ M which is stimulatory to growth. A higher concentration of $2.9 \times 10^{-4}$ M, however, was found to reduce amino acid incorporation in cucumber (Cucumis sativus L.) cotyledon discs. These results were in agreement with the data of Bugg and Hayes (42) who also reported inhibition of protein synthesis in excised corn coleoptile tips with mefluidide at $2.9 \times 10^{-4}$ M concentration. This $2.9 \times 10^{-4}$ M concentration, although, was inhibitory to incorporation of $^{14}$C-leucine into protein, but absorption of $^{14}$C-leucine was unaffected (267). These authors (267) reported a requirement of pre-treatment lag period before the effects on incorporation of $^{14}$C into protein could be detected. They postulated that the effect of mefluidide on this system is indirect affecting DNA-directed RNA synthesis which in turn affects the rate of protein synthesis. At high concentrations, mefluidide may alter this nucleic acid metabolism resulting in inhibition of protein synthesis which could not be overridden by the application of GA$_3$ in corn (266). This hypothesis is supported by the findings of Briscovich (39) who determined that mefluidide affects protein synthesis at some stage(s) prior
to RNA synthesis using various inhibitors of m-RNA and t-RNA. The stimulation of protein synthesis may explain increased wheat (*Triticum aestivum* L.) yields with low rates of mefluidide (185).

Mefluidide, at high concentrations, was reported not to affect either state 3 or state 4 respiration in isolated mitochondria (267). A concentration of $1 \times 10^{-4}$M mefluidide also did not affect photosynthesis of cucumber cotyledon discs over a 24 h period (267). Studies with intact corn plants showed that mefluidide at $2.9 \times 10^{-4}$M concentration caused shoot-growth reduction of the plants accompanied by some distortion and chlorosis of the bases of those leaves formed soon after treatment. This observation was correlated with a decrease in chlorophyll $a$ content. Based on the results of photosynthesis and respiration studies, Truelove et al. (267) proposed that the observed affects on protein synthesis are probably not due to changes in ATP levels. Perfluidone {1,1,1-trifluoro-N-[2-methyl-4-(phenylsulfonyl)phenyl]methanesulfonamide} (Figure 3), a mefluidide-related herbicide, however, has been shown to uncouple photophosphorylation by affecting the ATPase activity of isolated chloroplasts of spinach (*Spinacia oleracea* L.) (2).

St. John and Hilton (250) reported an inhibition of synthesis of neutral and polar lipids in vitro due to perfluidone. This indicates that an alteration of membrane structure and permeability in plant cells could be the site of perfluidone action. Tafuri et al. (260) showed enhancement in the activity of IAA-oxidizing enzymes in *Lens culinaris* roots. They postulated that an increase in the degradation of IAA through enhanced activity and/or synthesis of IAA-oxidase may be the mechanism resulting in an alteration of the growth pattern of plants.
Glenn et al. (113) determined the association of $^{14}$C-label of mefluidide with various organelles of cells from treated-corn leaf using the differential centrifugation technique. Less than 3% of the label that remained in treated leaf was found to be associated with nucleus, chloroplasts, mitochondria, and ribosomes. Ninety percent of the label was found to remain with supernatant which consists mainly of soluble enzymes and cytoplasm.

E. Uptake and Translocation of Mefluidide

Uptake, translocation, and metabolism of mefluidide in selected species have been studied using $^{14}$C-labelled herbicide in order to elucidate the mechanism of its selectivity. These studies in many cases, however, failed to explain the basis for differential responses of mefluidide-treated species.

The influence of environmental factors on uptake and translocation of mefluidide in several tolerant and susceptible species has been investigated by several workers. McWhorter and Wills (183) studied the influence of temperature and relative humidity (RH) on the absorption and translocation of $^{14}$C-mefluidide in tolerant soybeans and susceptible common cocklebur and johnsongrass. At a constant level of 40 or 100% RH, an increase in air temperature from 22 to 32 C resulted in 2- to 3-fold increase in absorption and 4- to 8-fold increase in translocation of $^{14}$C in soybeans following application to the second trifoliolate. An increase in RH from 40 to 100% resulted in <2-fold increase in absorption or translocation at a constant temperature of 22 or 32 C. In cocklebur, $^{14}$C absorption increased about 3-fold at both 22 and 32 C with an increase in RH from 40 to 100% following application to the second alternate leaf. At both levels of RH, absorption and translocation were
increased as the temperature was increased from 22 to 32 °C. When 14C-
mefluidide was applied to johnsongrass, the absorption increased 5- to 6-
fold at both 22 and 32 °C as RH was increased from 40 to 100%. Transloca-
tion in johnsongrass was affected less by variations in temperature than
it was in soybeans or common cocklebur. Addition of a surfactant was
found to increase absorption and translocation of 14C under all climatic
conditions in common cocklebur, but only under varying RH in johnsongrass.
These results are in agreement with previously published reports of Wills
and McWhorter (280) who reported that the absorption of 14C-mefluidide
was increased at 72 h when temperature was increased from 22 to 32 °C at
a constant RH of 100% with a similar pattern of distribution at each
temperature both in soybeans and common cocklebur. From further investi-
gations, Wills and McWhorter (281) summarized that there was a greater
absorption and translocation of 14C-mefluidide at 32 °C and 100% RH than
at 22 °C and 40% RH in soybeans, common cocklebur, and johnsongrass. These
observations point out that absorption of 14C-mefluidide was RH-dependent
in johnsongrass and temperature-dependent in soybeans.

In general, there are differences in the amount of uptake and trans-
location of 14C-mefluidide within a species depending on the plant part
to which the 14C-label was applied. Wills and McWhorter (280) found
that 35 to 45% of the herbicide was absorbed when label was applied to
stems or leaves of soybeans, whereas the absorption was 70 to 90% when
label was placed on apical bud. Translocation of 14C was 30 to 35% from
stems, 15 to 20% from leaves, and <10% from the bud. Further, the trans-
located label was 3 to 4 times greater from stem-application than when
label was applied to the leaves. The same trend was also noted with
common cocklebur. Absorption and translocation of 14C-mefluidide from
root applications were reported to be much lower than from shoot applications (30,31,32). One might expect a higher absorption and a lower translocation of the label from bud-applications as compared to applications to other plant parts, if the bud serves as a sink for this herbicide.

In several species studied, there seems to be a greater uptake and translocation of mefluidide in susceptible species as compared to tolerant species. Wills and McWhorter (280) reported a greater uptake and translocation of \(^{14}\)C-mefluidide in common cocklebur as compared to soybeans. Greater absorption of \(^{14}\)C-mefluidide was also reported in johnsongrass as compared to soybeans in another study (281). But Bloomberg and Wax (31) obtained a greater shoot (52.1%) and root (22.7%) uptake of \(^{14}\)C-mefluidide by tolerant soybeans as compared to the uptake by shoot (40.1%) and root (17.1%) of susceptible common cocklebur at the end of eight days. In separate studies, Bloomberg and Wax (32) observed that the rate of leaf absorption and translocation of \(^{14}\)C-mefluidide to be initially greater in highly susceptible giant foxtail than moderately susceptible common cocklebur or tolerant soybeans. Yet no increase in absorption or translocation of the label with time was observed in giant foxtail. At eight days, absorption and translocation were reported to increase in soybeans and common cocklebur with slightly greater amounts in the former. Common cocklebur was reported to translocate 47% more label than soybean out of the treated leaf. Soybeans and giant foxtail were found to exude mefluidide from roots into the nutrient solution, whereas this exudation was not noted with common cocklebur. Based on these results, Bloomberg and Wax (31,32) attributed the susceptibility of giant foxtail to rapid uptake, translocation, and
accumulation of the label in meristematic tissue. The increased susceptibility of common cocklebur was attributed to increased translocation of label out of the treated leaf and accumulation in the meristematic regions. These results with common cocklebur do not agree with those of Wills and McWhorter (280) possibly because of differences in their methods such as uptake period, temperature, and relative humidity. Glenn and Rieck (110,111) observed an increased uptake and translocation of foliarly-applied $^{14}$C-mefluidide with time only in tall fescue but not in johnsongrass or shattercane. The label was reported to be translocated throughout the entire plant including root, stem, and upper and lower leaves.

In these uptake and translocation studies, there are considerable variations in the rates at which the label was reported to be absorbed and translocated by the susceptible grass species. These variations could be attributed to differences in uptake periods and rates of mefluidide application. Bloomberg and Wax (32) followed the uptake and translocation of the label up to 8 days in giant foxtail. They did not observe any increase in these two processes with time, probably because of the injury caused by high rates of mefluidide immediately following the application. Glenn and Rieck (111) determined the uptake and translocation of the label in tall fescue up to 6 days and found an increase in the label inside the plant with time, but they used only sub-lethal concentrations of mefluidide. In one study of Glenn and Rieck (110), the uptake and translocation of the label was determined at 1 and 24 h. This period is considerably shorter than that of Bloomberg and Wax (32), and because uptake was determined only twice during the course of the experiment, the rates of these processes could not be determined accurately.
Schweizer and Eshel (242) also reported differences in the amount of $^{14}$C-mefluidide translocated in tolerant and susceptible species which support the data of Bloomberg and Wax (32). At 25 days after treatment, 77% of the label was found to move out of the treated leaf in wild mustard, a susceptible species as compared to 51% movement in intermediate redroot pigweed and sugarbeet, and <25% translocation in tolerant common lambsquarters, barnyardgrass, and wild oats. The highest concentration of $^{14}$C was found in the apex of wild mustard whereas common lambsquarters and redroot pigweed had very little label in their apices. The grass species were reported to have highest concentrations of the label in the treated leaves (242).

In several of the species examined, $^{14}$C-mefluidide was reported to move primarily acropetally (31,32,110,111,183,242,280), however, some basipetal movement was noted (31,32,110,111,280) especially when the label was applied to the lower 1/3 of soybean stem (280). This translocation pattern suggests that shoot-applied mefluidide moves primarily in the phloem along with assimilate stream to newly formed leaves and areas of high metabolic activity (30,31,32,280) while the root-absorbed mefluidide moves primarily with transpiration stream and distributes uniformly over the entire plant (30,31,32) with slightly more accumulation in older tissues than younger tissues (32).

The results of Wills and McWhorter (280) and Bloomberg and Wax (31) indicate differences in absorption and translocation of mefluidide in tolerant and susceptible species. But these differences could not be attributed to selectivity of mefluidide because their results do not agree on whether tolerant or susceptible species take up and translocate more herbicide. The more recent work of McWhorter and Wills (183) has
proved that the selectivity of the target species is not correlated with the amount of $^{14}$C-mefluidide absorbed or translocated. McWhorter and Wills (183) thus attributed the selectivity to differences in plant metabolism.

F. Metabolism of Mefluidide

Bloomberg and Wax (29,32) studied the metabolism of mefluidide in soybeans and common cocklebur by pulse labelling of $^{14}$C-mefluidide through petioles of excised leaves. The levels of free mefluidide rapidly decreased with time in soybeans. After 72 h, only 56% of the label in leaf extract remained as mefluidide, <5% was converted to two metabolites, MBR-12130 and MBR-15733, and 39% was found to be associated with the water-soluble fraction. The label that could not be extracted (bound portion) constituted 13.5% of the total label present in the leaf. Acid hydrolysis followed by organic solvent extraction removed only 30% of the label that was found in water-soluble fraction. Of the extracted water-soluble fraction, free mefluidide, MBR-12130, and MBR-15733 represented 74, 6.8, and 6.5%, respectively. Bloomberg and Wax (29,32) indicated that mefluidide and its metabolites may form acid-labile conjugates in the polar fraction of tolerant soybeans. In common cocklebur, mefluidide metabolism was relatively slow. After 72 h, free mefluidide and the label in the water-soluble fraction of the total label in the leaf extract represented 92 and 6.7%, respectively. The bound portion was only 4.4% of the total label in the leaf. Only traces of MBR-12130 and MBR-15733 were found in the extract. Based on these observations, these authors proposed a scheme in which tolerant soybeans dissipate mefluidide throughout entire plant, exude some of the herbicide through roots, rapidly metabolize mefluidide, conjugate mefluidide and its metabolites,
form water-soluble products, and bind the label within the leaf. The susceptible common cocklebur only slowly metabolizes mefluidide to its less toxic compounds. Bloomberg (30) also studied the causes for differential responses of soybean cultivars to mefluidide. Tolerant cultivars were reported to translocate less label out of treated leaf and metabolize more mefluidide to water-soluble products as compared to susceptible cultivars.

Glenn (42) also obtained results similar to those reported by Bloomberg (30). Glenn (108) used hypocotyl sections of soybeans and corn to study metabolism of mefluidide. Soybeans metabolized 47% more ^14C-mefluidide as compared to susceptible corn. Soybean extract had greater amounts of label remaining at origin of thin-layer chromatography (TLC) plates as compared to corn. Small amounts free MBR-12130 and MBR-15733 were also present in the extracts of soybeans whereas these two compounds could not be detected in the extracts of corn. The label that remained close to origin on TLC plates was polar. Acid hydrolysis of this material from both the species was also found to release 23% free mefluidide. Glenn (108) suggested that mefluidide may have conjugated with a natural plant constituent at a faster rate in soybeans as compared to corn constituting a possible basis for mefluidide selectivity. The presence of a sugar in the mefluidide conjugate was confirmed by Bloomberg (30).

G. Mode of Action of Mefluidide-Related Compounds

The amide herbicides are usually applied to the soil for preemergence control of weeds. Propanil, solan, and mefluidide, however, are more active when applied postemergence to the target species.
I. Morphological Responses of Plants

Hussain (134) studied the morphological responses of barnyardgrass, yellow foxtail, red rice, and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] to postemergence applications of alachlor, metolachlor, butam, and diethatyl. The responses of these grasses include height reduction, development of callus growth at the base of the plant near soil level, and necrosis starting at leaf tips and proceeding toward the base of the plant. In soybeans, only height reduction and crinkling of leaves, however, were observed. Although these herbicides are normally applied preemergence to weeds, the morphological responses of these grasses due to early postemergence applications are similar to those caused by the postemergence application of mefluidide.

II. Growth Responses

Several of these amide herbicides are known to inhibit plant growth in general by affecting one or more plant processes. Alachlor (41,67,144), diphenamid (69), pronamide (47), and foliarly-applied propanil (133) were reported to cause general inhibition of root growth and development. The herbicides that specifically inhibit root elongation include CDAA (46), propachlor (79), alachlor, diethatyl, metolachlor (35), napropamide, diphenamid (89), and propanil (133). Keeley et al. (144) observed inhibition of lateral root development in seedlings due to soil applications of alachlor. Inhibition of cell division due to CDAA (46), diphenamid (69), alachlor, and metolachlor (67) and cell enlargement due to alachlor and metolachlor (67) were also reported to be the main factors in causing decreased growth of grasses as well as broadleaf plants. Carlson et al. (47) investigated the influence of pronamide on various phases of cell division in roots. They found that pronamide
arrested mitosis in the metaphase causing multinucleate cells.

These amide herbicides were also found to inhibit shoot growth in general either directly (41, 46, 47, 89, 133, 144) or indirectly by inhibiting root growth (69).

III. Biochemical and Physiological Responses

Chloroacetamides, in general, appear to inhibit protein synthesis without any exception (67) although this may not be the primary mechanism of phytotoxicity. Inhibition of protein synthesis by propachlor in root tips (79), CDA in barley (Hordeum vulgare L.) and hemp sesbania (169), propanil in half barley seeds (194), metolachlor in cucumber (64), and alachlor in deembryonated barley seeds (232) and shoots and roots of wheat (53) was clearly demonstrated. Moreland et al. (194) observed decreased RNA synthesis by propanil in half barley seeds which indicates that propanil exerts its effects on protein synthesis at same stage(s) prior to RNA synthesis. Rao and Duke (232) reported that inhibition of protein synthesis due to alachlor was caused through inhibiting GA-induced production of protease and α-amylase in deembryonated barley seeds. However, subsequent research by Duke et al. (79) with propachlor in cucumber hypocotyl sections indicates that this was not the case. Duke et al. (79) postulated that inhibition of protein synthesis was due to prevention of auxin-induced enzyme formation.

Metolachlor was reported to inhibit lipid synthesis in root tips of cotton. Reduced synthesis of total lipids and complete inhibition of incorporation of choline into phosphatidyl choline have been demonstrated by Diner et al. (71) and Truelove et al. (268).

The inhibition of protein and lipid synthesis indicates that these amide herbicides may affect membranes. Pillai et al. (222, 223) proposed
that metolachlor causes structural and functional alterations in plant membranes from their investigations with the roots of onion (Allium cepa L.), cucumber, and cotton. This hypothesis was recently supported by the findings of Deal and Hess (67) who also reported membrane leakage due to metolachlor. Propanil also appears to destroy chloroplast membranes and increase the permeability of red beet (Beta vulgaris L.) membranes (133).

The other physiological processes affected by these herbicides include respiration, photosynthesis, and transpiration. Respiration was reported to be inhibited by CDA in pine (Pinus resinosa L.) seedlings (240) and by propanil in lambsquarters (133). Gruenhagen and Moreland (117) also reported inhibition of oxidative phosphorylation and reduced ATP production in excised soybean hypocotyls due to propanil.

Moreland Hill (193) reported inhibition of the Hill reaction in isolated chloroplasts by propanil. Nishimura and Takamiya (205) later specified the site of action as the inhibition of cytochrome 553 reduction by photosystem II. Solan, another postemergence herbicide, was also reported to cause inhibition of photosynthesis both in resistant tomatoes (Lycopersicon esculentum Mill.) and susceptible egg plant (Solanum melongena L.); but tomatoes recovered rapidly whereas egg plant photosynthesis continued to decline. Light appears to be a requirement for solan injury, as is the case with most other photosynthetic inhibitors (58). The research of Chandler et al. (53) indicates that the Hill reaction with chloroplasts of wheat, however, was not affected by alachlor. There seems to be a relationship between the presence of a free and sterically unhindered amide or imino hydrogen and the activity of acylanilides that inhibit photosynthesis (13). One report showed inhibition of transpiration by propanil (243).
Bucholtz and Lavy (41) found decrease in shoot and root growth of susceptible oats (Avena sativa L.) and tolerant soybeans by alachlor. They observed a direct correlation between root growth and the amount of $^{32}\text{PO}_4$ and $^{35}\text{SO}_4$ in shoots of both the treated species.

IV. Uptake and Translocation

Most of the amide herbicides are reported to be absorbed primarily by the root and translocated to the shoot. Some herbicides, however, are also known to be taken up by the shoot before the seedlings emerge from the soil. CDAA (140) and diphenamid (159) were reported to be readily absorbed by roots of the seedlings from preplant incorporated or preemergence applications. Jaworski (140) found that root absorbed CDAA was readily translocated to the shoot. Nishimoto et al. (204) and Knake and Wax (147) demonstrated that propachlor was taken up more by the shoot than by the root from soil. Foliar applications of propanil, a contact herbicide, was reported to result in only limited translocation (289).

Metolachlor is known to prevent emergence of seedling grasses and broadleaf weeds (67). Diner et al. (70) studied uptake and translocation of soil-applied metolachlor. Soil-applied metolachlor was taken up by the root, translocated throughout the plant, and accumulated in older leaves. The foliar applications were found not to cause any inhibition (64) except for necrosis at the site of placement probably because of limited translocation from this foliar application (70).

Alachlor is a selective preemergence herbicide used for the control of several annual grass and broadleaf weeds in corn, peanuts, and soybeans (52) and is known to inhibit the emergence of the weed seedlings (67). Knake and Wax (147) showed alachlor to be more effective when
placed in the shoot zone as compared to seed zone. Bala Narasaiah and Harvey (20) studied the selectivity of soil-applied alachlor to corn planted at various depths. Exposing shoot (mesocotyl or first internode plus coleoptile and plumule) was found to be more sensitive than seed or root. Deeper planting of corn was reported to give protection to the crop as the sensitive shoot zones were not in immediate contact with surface-applied alachlor during corn emergence. Armstrong et al. (11) also specified the main site of uptake of alachlor as the portion of the plant above the tuber in 2 to 4 cm-tall yellow nutsedge. The translocation of alachlor seems to be primarily acropetal in direction. Root-applied alachlor was reported to translocate throughout the plant whereas foliar-applied chemical moved apoplastically only within the treated leaf both in resistant soybeans and susceptible wheat. Wheat had greater uptake and translocation as compared to soybeans although both species were shown to accumulate more $^{14}$C in older leaves as compared to younger leaves. Light seems to play an important role in the amount of $^{14}$C absorbed with greater absorption in the light than in the dark (52). Armstrong et al. (11) demonstrated some basipetal movement of shoot-applied alachlor although most of the alachlor was translocated primarily in acropetal direction in young yellow nutsedge.

Deli and Warren (69) showed that root application of diphenamid caused both root and shoot growth reduction of oat seedlings primarily because of root uptake. They also observed some shoot uptake which did not result in any growth reduction. These findings were supported by the results of Hodgson and Hoffer (132) who also observed extensive root uptake of diphenamid by pepper (Capsicum frutescens L.) from nutrient solution. Diphenamid also appears to translocate apoplastically in test
species such as tomatoes (28) and pepper (132).

Pronamide is widely used for grass and broadleaf weed control in agronomic and horticultural crops (270). Pronamide was found to be most toxic when placed in the seed zone of oats (48), although it did not affect seed germination (47). Soil-application of pronamide led to root uptake and translocation whereas foliar-application did not result in any phytotoxic actions because of lack of uptake in established oats (48).

V. Metabolism

The mechanism of selectivity of amide herbicides were not only attributed to differences in uptake and translocation, but also to the inactivation of the toxicant in tolerant species.

Jaworski (140) reported degradation of CDAA to glycolic acid and probably to diallylamine which could eventually enter metabolic pathways in tolerant plants.

Long et al. (160) studied the mechanism of action of diphenamid and concluded from their studies that diphenamid was accumulated in shoot where it was metabolized extensively in tobacco seedlings. The roots were found to contain only low amounts of the herbicide where the metabolism was relatively slow. These results suggest that tolerant species probably translocate diphenamid to the regions where it could be metabolized at a faster rate while susceptible species fail to do so. Davis et al. (65) isolated three major metabolites along with two polar metabolites of diphenamid all of which were conjugated to glucose.

Carlson et al. (48) observed pronamide to be adsorbed to cell walls of treated roots of susceptible oats where it stopped normal mitosis at metaphase.
The metabolism of propanil has been studied more extensively than any other amide herbicide. The main pathway of degradation seems to be hydroxylation followed by complexing with glucose and lignin (289).

Porter and Jaworski (224) reported propachlor degradation in corn and soybeans to be very rapid since no propachlor was detected even at the earliest harvest following the treatment. Analysis of the metabolite suggested that this chloroacetanilide was conjugated through its active chlorine to some natural product to form a glycosidic linkage. This chloro group appears to have been displaced by some nucleophilic endogenous substrate making this metabolite relatively nonphytotoxic. Lamoureux et al. (154) later showed propachlor to be conjugated with glutathione in corn seedlings and excised leaves of corn, sorghum, sugarcane, and barley. They also isolated two more conjugates one of which was suggested to be γ-glutamyl cysteine conjugate of propachlor.

In conclusion, the mechanism of action studies on the amide herbicides have not yielded a consistent pattern. The plant processes that are affected by one or more of these amide herbicides include photosynthesis, respiration, transpiration, RNA synthesis, protein synthesis, lipid synthesis, mitosis, and enzyme production.

H. Physical and Chemical Properties of Bentazon

Bentazon is an odorless, white crystalline solid with a molecular weight of 240.3, a melting point of 137-139 °C, and decomposition temperature of 200 °C. Bentazon is readily degraded in ultraviolet radiation (200-400 nm), but not in visible light (400-600 nm). Its vapor pressure is \(<10^{-8}\) mm Hg at 20 °C (270). Bentazon is relatively insoluble in water (500 mg/L) and cyclohexane (200 mg/L), but is highly soluble in acetone (\(1.5 \times 10^6\) mg/L), ethanol (\(8.61 \times 10^5\) mg/L), ethyl acetate (\(6.5 \times 10^5\) mg/L), ether (\(6.16 \times 10^5\) mg/L), and chloroform (\(1.8 \times 10^5\) mg/L).
Bentazon is formulated as a water-soluble sodium salt containing 480 g bentazon per liter and supplied under the trade name, "Basagran®" (17,270). The structure of this compound is shown in Figure 4.

I. Activity and Selectivity of Bentazon

The herbicidal properties of bentazon for the control of broadleaf weeds in crops of the grass family were first described by Fischer (96) during the ninth British Weed Control Conference in 1968. Bentazon was discovered to be a potential broadleaf herbicide in soybeans in 1970 during field trials in Mississippi (3). BASF Wyandotte Corporation started a development program and obtained an experimental use permit in 1973 to evaluate bentazon for weed control mainly in soybeans (3). Since then, it has become one of the most successful new herbicides used by soybean farmers in the United States. Now bentazon is registered for use in soybeans, peanuts, green and dry beans (Phaseolus vulgaris L.), green and dry peas (Pisum sativum L.), corn, rice, and in established crops like peppermint (Mentha piperita L.), spearmint (Mentha spicata L.), and variety of ornamental turfgrasses (3,18). Luib and Weerd (161) tested bentazon in rice throughout the rice growing areas of the world and found wide range of broadleaf weeds to be susceptible which are normally resistant to phenoxy herbicides.

I. Weed Control

Bentazon is recommended as a selective postemergence herbicide for broadleaf weed control. Bentazon does not control grasses and has negligible preemergence activity (17,18). Amsberg (3) indicated that for effective weed control, the susceptible weeds should have no more than 6 true leaves and no taller than 8 to 10 cm. Since it is not a readily translocated herbicide, proper coverage of the weeds is necessary for
Figure 4. Structural formula of bentazon [3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide].

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effective control (3). The directions on the label (18) indicate that applications of bentazon should be made to weeds at the 2 to 6 true-leaf at rates of 0.84 to 2.24 kg/ha for the control of most weeds except for yellow nutsedge and Canada thistle [Cirsium arvense (L.) Scop.]. Split applications of bentazon are recommended for the control of yellow nutsedge (27,87,126,130,145,293) and Canada thistle (38,130) at 0.84 plus 0.84 kg/ha with the first application at the 15 to 20 cm-stage and the second 10 to 14 days later. Kinsella et al. (146) and Mathis and Oliver (173) emphasize that the stages of weeds, even at early growth periods, was very critical for effective control with bentazon. The addition of a wetting agent or a non-phytotoxic oil (2.3 L/ha) was suggested when bentazon was used on weeds partially controlled or on those not growing under favorable conditions (17,18).

There are numerous reports available from around the world on the activity and selectivity of bentazon on several crops and weeds as this herbicide has been sold commercially since 1975. Therefore, only a summary of the weeds controlled and the crops that showed tolerance will be presented here. Some of the weeds that are reported to be very susceptible to bentazon at normal rates (up to 1.12 kg/ha) of application are: 2- to 10-leaf (15 to 25 cm-tall) common cocklebur (3,5,15,18, 59,88,124,173,180,181,187,206,208,237,238,282,287,290,292,294), 2- to 5-leaf hemp sesbania (15,16,18,59,163,236,237,238), 20 cm-tall Canada thistle (18,88,130,208,276,282), Pennsylvania smartweed (Polygonum pensylvanicum L.) (5,15,18,59,88,187,208,282,292), morningglories (Ipomoea spp.) (18,88,173,237,287,292), pigweeds (Amaranthus spp.) (5,18, 173,187,196,282), prickly sida (18,88,173,236,237,238,287,292), common dayflower (Commelina communis L.) (15,18,59,62,88,97,98), wild common
High rates of bentazon (up to 2.24 kg/ha) were reported to be required for the effective control of tropic ageratum (Ageratum conyzoides L.), Alternanthera sessilis, Ammannia spp., waterhyssop (Bacopa spp.), Bergia agricata, eclipta [Eclipta alba (L.) Hassk.], hemp sesbania, waterprimrose (Jussiaea abyssinica) (161), Canada thistle (38), Scirpus maritimus (68), purple nutsedge (141), and cypressvine morning-gloxy (Ipomoea quamoclit L.) (263).

Only fair control of morningglories (124), pigweeds (124), and hemp sesbania (292) were reported at recommended rates (1.12 kg/ha) of application of bentazon.

The control of morningglories, however, was reported to be poor to excellent (178,236) because of differential responses of various species within this group even at early stages of growth. The differences in the control levels of various species of morningglories were attributed to the ability to metabolize toxic bentazon (178). Smallflower morning-gloxy [Jacquemontia tammifolia (L.) Griseb.], palmleaf morninggloxy (Ipomoea wrightii Gray), purple moonflower [Ipomoea muricata (L.) Jacq.] (18,178), pitted morninggloxy (Ipomoea lacunosa L.) (18,178,236), entireleaf morninggloxy [Ipomoea hederacea (L.) Jacq. var. integriuscula Gray], ivyleaf morninggloxy [Ipomoea hederacea (L.) Jacq.], and cypressvine morninggloxy (18) were found to be very susceptible, whereas other reports indicated that the control of tall morninggloxy [Ipomoea purpurea (L.) Roth] (236), entireleaf morninggloxy, and ivyleaf morninggloxy (178) was unsatisfactory with postemergence treatments of bentazon at recommended rates of applications at the 1 to 4 leaf-stage of weed development.

The negligible activity of bentazon at recommended rates (1.12 kg/ha) of application on grasses (18,161,237) and pigweeds (16,237), and at
half the recommended rates on hemp sesbania (104) were also reported. Soil applications of bentazon were found not to inhibit the germination of susceptible species such as radish (*Raphanus sativus* L.) seeds (190). It appears that bentazon is phytotoxic to only photosynthesizing plants.

The addition of a spray adjuvant to bentazon was reported to generally result in improved or more consistent control of several weeds. Lunsford et al. (162), however, observed that the addition of a spray adjuvant was not necessary to produce the control of labeled weeds, but would help in adverse environmental conditions. The beneficial effects on the control of a variety of weeds due to addition of a surfactant, alkylaryl polyglycol ether (Citowett Plus) (87,163) at 0.25% (v/v) or oil concentrate (At Plus 411 F) at 9.3 L/ha (15) to bentazon have been documented. Nalewaja et al. (198) found the addition of emulsifiable linseed oil formulation to bentazon to be effective in reducing the detrimental effects of rain and cold or dry weather, whereas water soluble linseed oil formulation was found to increase absorption and translocation of bentazon by reducing the effects of low relative humidity and old age of the leaves. Harader (121), on the other hand, found no advantage of adding a wetting agent to bentazon at 0.84 kg/ha on the control of 4-leaf (13 cm-tall) common sunflower. The environmental conditions were probably too conducive for toxic effects of bentazon applied alone on common sunflower to permit improvement.

Poor coverage of weeds such as yellow nutsedge (87,251) and common cocklebur (206) has been shown to result in loss of weed control due to contact action of bentazon. Oliver et al. (206) found that a spray volume of 281 L/ha gave better control of common cocklebur due to more thorough coverage as compared to a low volume of 94 L/ha.
II. Crop Selectivity

The crop species that were reported to be tolerant to bentazon at recommended rates (up to 1.12 kg/ha) of application include winter wheat, spring barley (*Hordeum vulgare* L.) (184), rice (187,188), corn, and barley (187), and several turfgrasses including Kentucky bluegrass (27,137,145). Soybeans (23,208,275,287), peanuts (88), rice (59,68), corn (88), peppermint (38), and variety of turf grasses (141) were tested to be tolerant to bentazon even at high rates (2.2 to 4.4 kg/ha) of application.

Although most tested soybean cultivars are tolerant to bentazon, there are a few cultivars such as Hurrel brink (275), PI 243.532, and PI 229.342 (127) which were found to be sensitive. Only slight soybean injury, however, was noted with high rates (2.2 kg/ha) of application of bentazon. Soybeans were found to develop slight general chlorosis with a few necrotic spots which later disappeared. No losses in soybean yields were reported due to injuries caused by bentazon (18,236,287). Increase in soybean (180,237,287) and rice (68) yields due to control of several troublesome weeds with bentazon were recorded.

Dowler and Parker (77) made detailed investigations on the influence of bentazon (0.84 kg/ha) on various yield components of soybean and found that bentazon sprayed at 18, 36, or 54 days after planting did not affect any of the components studied including height, lodging, stand, pod set, seed quality, weight/100 seeds, or yields.

J. Influence of Environmental Factors on the Activity of Bentazon

Environmental factors such as rainfall, temperature, relative humidity, presence of dew, light intensity, applications at various times of the day, and soil moisture; and plant factors such as leaf orientation
are found to significantly affect weed control and crop selectivity
due to bentazon applications.

I. Rainfall

Andersen et al. (5) observed reduced effectiveness of bentazon on
selected weeds in several instances when rain occurred soon after the
treatment. Doran (73) and Doran and Andersen (74) investigated the
influence of simulated rain on the activity of bentazon on common
cocklebur and velvetleaf and found that rain within 8 h after herbicide
application resulted in loss of activity in the greenhouse. In the
fields, however, the reduced activity was noted with simulated rain up
to 24 h on velvetleaf and even >24 h on cocklebur. The differences be­
tween greenhouse and field results were attributed to differences in
other environmental factors such as light intensity and duration, tem­
perature, and RH between greenhouse and fields.

Nalewaja et al. (201) also reported reduced pigweed control due to
simulated rain within 24 h after bentazon application. They observed
that a heavy rain (1300 L/ha) within 1.5 h after bentazon application
decreased the control of pigweed due to washing the herbicide off of the
plant, while a light rain (650 L/ha) increased the control by rewetting
the dried herbicide on the leaf surface (200,201). The effectiveness of
rainfall, however, was reported to be lowered either by adding a spray
adjuvant such as linseed oil (73,74,196,198,200,201) or a surfactant
(5,74) which were found to increase the spray retention or just by in­
creasing the rates of bentazon (5).

II. Relative Humidity

Relative humidity is one of the factors that would influence the
physical state of the herbicide on plant surface. The longer the herbi-
cide is in liquid form, the greater the uptake would be. High RH (96%) (277,278) was reported to give better control of pigweed (200,201), common cocklebur (164,277,278), and purple nutsedge (278) as compared to low RH (30 to 40%) due to bentazon treatments. The addition of an oil additive (196,200,201) or a surfactant (164) was found to overcome the effects of low RH (196,277,278) probably due to increased absorption (201).

III. Temperature

Temperature appears to play an important role in determining not only the speed but also the effectiveness of weed control. Nalewaja et al. (196,200,201) reported an increase in the control of redroot pigweed as the temperature was increased from 10 to 30 C. Similar observations were also made on purple nutsedge (278) and common cocklebur (277,278) when there was an increase in temperature from 25 to 35 C. Suwanketnikom and Penner (252) reported significantly better control of yellow nutsedge at 25 and 35 C than at 15 C. Orr et al. (208) determined that the day and night temperatures of below 26.7 and 15.6 C, respectively, were not conducive for weed control with bentazon. Wills and McWhorter (279), from their studies with 14C-labelled bentazon, attributed this increase in weed control to increased translocation of bentazon at high temperatures.

IV Light Intensity

Potter and Wergin (226) found that the higher the illuminance the faster the development of necrotic flecks on bentazon-treated cocklebur. Since bentazon is a photosynthetic inhibitor, it should not be surprising to see a direct correlation between light intensity and severity of toxic symptoms. Yellow nutsedge, however, did not respond the same way
as cocklebur did. Exposing bentazon-treated yellow nutsedge (7.6 cm-
tall) plants to a low light intensity (16146 lux) was found to result in
more damage as compared to a high intensity (48438 Lux) (252). At high
intensity, bentazon probably caused a localized necrosis of the treated
leaves due to its contact action and in such cases plants could partially
recover from the injury utilizing reserved carbohydrates from their
tubers. A low light intensity might have caused a depletion of the
reserved carbohydrates from underground tubers as well as a slow and
steady translocation of bentazon to the meristematic tissues which al­
together might have eventually led the plant to its death.

V. Day Versus Night Applications

Investigations on the basic mode of action of bentazon strongly
indicate a requirement of light for this herbicide to exhibit its phyto­
toxicity in susceptible weeds. With consideration of this possibility,
several researchers were interested in investigating the differences,
if they exist, between day and night applications of bentazon under
grower conditions. Lunsford et al. (165) did not find any differences
in the control of several weed species in 17 tests due to bentazon appli­
cations at 7 a.m., 1 p.m., or 12 midnight. They, however, noted that the
control of velvetleaf was better in day applications when the plants were
under drought stress conditions caused by dry weather. Doran and Ander­
sen (75), on the other hand, reported poor control of cocklebur and vel­
etleaf due to bentazon applications at night, evening, or early morning.
They attributed this response to low temperatures and/or presence of heavy
dew in the fields during those periods as compared to midday applications.
In the greenhouse, Doran and Andersen (75) noted that the control of
cocklebur was unaffected by the timing of application, but velvetleaf
control was poor at night because the leaves were droopy and intercepted less herbicide due to nyctitropic leaf movement. These observations were substantiated by subsequent research of Aaersen and Koukkari (4) and Kraatz and Andersen (149) who also observed the leaves of velvetleaf to be horizontal during the day and droopy during the nights. These authors (4,149) proposed that the amount of spray intercepted and retained is a function of varying leaf angles.

VI. Soil Moisture

Soil moisture not only influences plant growth but also induces structural changes which contribute to altered tolerance of weeds to herbicides. Actively growing weeds, in general, are more susceptible to herbicides than those growing slowly under moisture stress conditions. Lunsford et al. (162) thus emphasizes good soil moisture for better weed control with bentazon. Unsatisfactory control of yellow nutsedge (87, 252) in peanuts (87) was reported due to drought stress conditions following bentazon applications. Wills and McWhorter (278) attributed increased control of purple nutsedge and cocklebur to increased translocation of $^{14}$C-bentazon under wet conditions as compared to dry conditions. Wills (277) also observed greater bentazon toxicity to cocklebur when soil moisture was increased from near the wilting point to field capacity. The soil applications of bentazon were also reported to be influenced by soil moisture with greater toxicity to Cyperus serotinus Rottb. and Sagittaria pygmaea Miq. under flooded conditions as compared to dry conditions (187).

Increased soybean injury, however, was noted due to bentazon applications under moist conditions (5,218,287) because of increased root uptake (5,219) and increased translocation from the site of uptake (279).
K. Uptake, Translocation, and Metabolism of Bentazon

The selectivity of bentazon between crops and weeds as well as among cultivars within a crop species are commonly attributed by various researchers to differences in uptake, translocation, and/or metabolism of the herbicide.

I. Basis for Selectivity of Bentazon

To summarize various studies on the basis for bentazon selectivity, the evidence points out that the tolerant species have one or more of the factors in their favor such as: i) less spray retention on leaf area basis (168,218,219), ii) less total absorption (128,171,197,234,296), iii) less translocation (128,168,171,188,197,234,277,279), iv) only acropetal translocation (167,218,279), v) faster and greater metabolism, degradation, or conjugation (45,63,128,151,166,167,168,188,189,190,191,210,211,212,214,218,219,233), vi) faster and total recovery from inhibition of photosynthesis (26,33,63,188,189,190,218,219,225,226,233,234,295), vii) rapid recovery from inhibition of respiration (177,218,219), and viii) temporary increase in the rates of transpiration (218,219). The susceptible species were reported to differ from the tolerant species at least in one of the above processes examined, although every single process alone might not have played a role in determining herbicidal action.

II. Influence of Environmental Factors on Uptake, Translocation, and Metabolism of Bentazon

Environmental factors were determined to have significant influence on the control level of weeds by affecting mostly uptake and translocation rather than metabolism of bentazon. The responses, however, seem to be species-dependent. Dannigkeit (63) reported an increase in the uptake
of bentazon at 30 C and 80% RH as compared to 11 and 20 C and 40% RH in
wheat, barnyardgrass, common cocklebur, false chamomile [Matricaria mari-
tima L. var. agrestis (Knaf) Wilmott], corn marigold (Chrysanthemum seg-
etum), and catchweed bedstraw (Galium aparine L.). But in rice and soy-
beans, the uptake was greater at 11, 12, and 20 C and 40% RH as compared
to 30 C and 80% RH. Nalewaja et al. (197), on the other hand, observed
more absorption and translocation of 14C by redroot pigweed as the tem-
perature and relative humidity were increased from 10 C and 35% to 30 C
and 100%, respectively. Also the young leaves appear to absorb more
label than the older leaves. They suggested the use of an emulsifiable
linseed oil to reduce the influence of low relative humidity and leaf
age upon 14C-bentazon absorption. The results of Wills and McWhorter
(279) also indicate that there is more translocation of 14C both in
tolerant and sensitive soybean cultivars at a high temperature (35 C)
than at a low temperature (24 C). Relative humidity appears to play a
significant role in determining the direction of translocation of 14C
in certain species tested. Wills (277) and Wills and McWhorter (278)
observed highest translocation of 14C in common cocklebur grown in wet
soil at 35 C and 96% RH. At 35% RH, the label was reported to move both
acropetal and basipetal in direction whereas at 96% RH, the translocation
was limited to only acropetal in direction. But in purple nutsedge, the
14C was found to translocate to immature leaves on the treated shoot and
to newly forming shoots through connecting rhizomes at 35 C and 96% RH.
When RH was lowered to 35%, the accumulation of the label was confined
to the base of the treated leaf without subsequent translocation. In
soybeans, the translocation of the label was determined to be downward
from the treated leaf and was more vulnerable to variations in tempera-
ture than to RH or soil moisture. The translocation of the label in soybeans was greater under wet soil conditions and high temperature than under dry soil conditions and low temperature (278).

Light intensity was also determined to influence the translocation of label in several susceptible species studied with more being translocated at a high light intensity as compared to a low light intensity (234).

Zaunbrecher and Rogers (296) reported no effects of soil moisture or plant stage on either absorption or translocation of label in tolerant soybeans or susceptible common cocklebur.

There is very limited literature available on the influence of environmental factors on the metabolism of bentazon. Dannigkeit (63) reported rapid metabolism of bentazon in catchweed bedstraw at 40% RH and low temperatures as well as at 80% RH and high temperatures than any other conditions studied. In other species, the conversion of bentazon was found to be dependent only on the speed of uptake.

III. Pathways of Uptake and Translocation

Bentazon appears to reach the plant interior via both lipophilic and hydrophilic pathways because the herbicide is available as lipophilic undissociated acid or as hydrophilic dissociated molecule. In general, the lipophilic form was more readily absorbed than the hydrophilic form through the intercellular spaces in plant tissues (234). Although bentazon is applied to the foliage of the target species due to its contact action, delayed injury to *Cyperus serotinus* Rottb. was noted from soil applications of bentazon. The delayed injury was reported to result from root uptake followed by translocation to the shoot, the process which requires time (187,188,190). Similar reports were also made.
by Bethlenfalvay et al. (26) who also observed a delay in the inhibition of CO₂ exchange rates in beans (*Phaseolus vulgaris* L.) due to bentazon applied to roots as compared to applications to the foliage.

The general trend indicates that ¹⁴C-bentazon translocates acropetally in tolerant species and both acropetally and basipetally in susceptible species (128,167,168,171,187,188,190,234,251,279). There are a few reports, however, that do not follow this general trend. Potter (225) observed inhibition of photosynthesis only in leaves acropetal to the point of herbicide application in common cocklebur, although this species is very susceptible to bentazon. Penner (218) also observed mainly acropetal translocation in treated soybean and Canada thistle plants.

Stoller et al. (251) observed a slow acropetal translocation of foliar-applied bentazon in yellow nutsedge. But they noted the death of parent tubers although there was no basipetal translocation. The mechanism by which the tubers were killed was not known. Brewster and Stanger (38) reported basipetal translocation of bentazon in Canada thistle leading to movement from treated shoot to untreated shoot connected by roots. Bentazon translocates acropetally in xylem (234) probably along with transpiration stream, but Penner (218) also noted some phloem movement in Canada thistle.

**IV. Selectivity Between Soybean Cultivars**

It is becoming a common practice to utilize sensitive and tolerant cultivars of the same species as test plants to elucidate the mechanism of selectivity of a herbicide. Selecting the cultivars of the same species would eliminate interspecies plant factors such as differences in size of the plant at the time of treatment, leaf area, morphological...
and anatomical characteristics, etc. which would normally interfere in such critical studies.

Hayes and Wax (127) reported from their initial studies that there were no differences between tolerant Clark 63 and sensitive PI 243.532 or PI 229.342 cultivars of soybeans in absorption, translocation, or metabolism of bentazon, although sensitive cultivars exhibited visual toxicity symptoms in screening studies. There was no translocation of the label out of the treated central leaflet of 1st trifoliolate and all the label in the plant was reported to remain as bentazon in both types of cultivars. Hayes and Wax (127) reported no differences in the rates of photosynthesis as measured by oxygen evolution between the tolerant and sensitive soybean cultivars. Two years later, Hayes and Wax (128) reported a two-fold greater absorption and slightly greater translocation of the label in sensitive soybean cultivar, PI 229.342 than in tolerant Clark 63. Clark 63 was also found to metabolize bentazon at a more rapid rate than PI 229.342. Clark 63 contained two unidentified polar metabolites I and II, whereas PI 229.342 had only metabolite II in its system. Metabolite I was found to be labile to 6-glycosidic activity whereas metabolite II was unaffected. But both cultivars equally lost their photosynthetic activity by 50% as measured by oxygen evolution from isolated chloroplasts. Hayes and Wax (128) concluded that the differential responses of soybean cultivars are due to differences in absorption, translocation, and metabolism. They postulated that PI 229.342 does not have enzyme system to produce probably nontoxic metabolite I, and has only depressed enzyme system to produce metabolite II as its formation was too slow. The disagreement between the two reports of Hayes and Wax (127,128) could be due to
differences in their experimentation. In the first report, these authors used leaf discs, ultra low rates of bentazon, and shorter uptake periods whereas in the second report, intact plants, field rates of herbicide application, and longer uptake periods were employed.

Wills and McWhorter (279) also studied differential responses of two soybean cultivars to bentazon. They found that the tolerant cultivar, Hill, translocated less label and the translocation was mainly in acropetal direction as compared to sensitive Hurrelbrink which translocated more label, both in acropetal and basipetal direction, and into the roots. Although an increase in temperature from 24 to 35 C increased the translocation of the label, the increase was equal in proportion in both the cultivars. Utilizing $F_2$ crosses of Hill and Hurrelbrink in their subsequent research, Wills and McWhorter (279) concluded that the selectivity was based on differential translocation of the label and the translocation of the herbicide is genetically controlled in soybean cultivars.

The basis for selectivity of soybean cultivars can not be established from the above studies as there is no agreement between the results of Hayes and Wax (128) and Wills and McWhorter (279). The disagreement in their conclusions could be due to differences in their experimental techniques and selection of soybean cultivars.

V. Selectivity Between Soybean and Navy Bean (Phaseolus vulgaris L.)

Both soybeans and navy beans (94) are tolerant to bentazon applications, however, injury to navy beans having only unifoliolate leaves has been observed in the fields (167). This has created enough interest among researchers to study the causes for the susceptibility of unifoliolate- and the tolerance of trifoliolate-navy beans to bentazon.
Trifoliolate soybeans were included in these studies for comparison. Mahoney and Penner (166,167) found that foliar-applied $^{14}$C moved primarily acropetally in both species with some basipetal movement. The unifoliolate navy bean was found to translocate label both in acropetal and basipetal directions, whereas trifoliolate navy bean translocated label only acropetally from the treated central leaflet. The same number of methanol-soluble metabolites were found both in unifoliolate and trifoliolate navy beans. However, the percentage of unmetabolized bentazon was significantly higher in unifoliolate as compared to trifoliolate navy bean. The increased translocation of label downward and slow metabolism of bentazon might explain the susceptibility of navy bean at unifoliolate stage. Trifoliolate soybeans were found to have both acropetal and basipetal translocation. Soybeans translocated more $^{14}$C and metabolized a smaller percentage of bentazon than trifoliolate navy beans. This indicates that additional factors, other than translocation, contribute to the tolerance of soybeans to foliar applications of bentazon. The four apparent $^{14}$C-conjugates isolated from soybeans and navy beans were reported to be similar in nature.

Mahoney and Penner (168), from further investigations, attributed the tolerance of trifoliolate navy beans to less retention of bentazon on the leaf surface on a leaf area basis, limited translocation which was confined only to acropetal direction, and rapid metabolism. They concluded that greater spray retention on leaf area basis, extensive translocation both upward and downward, and slow metabolism all might have contributed to the susceptibility of unifoliolate navy beans.
VI. Selectivity Among Trifoliolate Navy Beans, Black Nightshade
(Solanum nigrum L.), and Common Cocklebur

There are numerous reports in the literature attempting to eluci­
date the mechanism of selectivity between tolerant crops and susceptible
weeds to foliar-applied bentazon. Most reports agree that the mechanism
of selectivity is based on differences in uptake, translocation, and/or
metabolism in tolerant and susceptible species. Because of the limita­
tions of space, the review on the basis for bentazon selectivity will be
limited to a few selected species such as tolerant trifoliolate navy
beans, moderately susceptible black nightshade, and susceptible common
cocklebur.

The leaves of common cocklebur and black nightshade were found to
retain more bentazon on the surface on an area basis than was retained
on the trifoliolate leaf of navy bean (168,218,219). The $^{14}C$ movement
in black nightshade seedlings was determined to be much greater than in
either common cocklebur or navy bean, and was found to translocate
throughout the entire plant. Since the extent of translocation does not
appear to be related to tolerance, Mahoney and Penner (168) believe that
the translocated label is not $^{14}C$-bentazon, but rather, readily trans­
located $^{14}C$-metabolites not found in either navy bean or cocklebur. In
cocklebur, the $^{14}C$ was reported to diffuse acropetally throughout the
entire leaf with accumulation near the leaf apex.

Bentazon metabolism in black nightshade was reported to differ
markedly from that in navy bean (168). There were a greater number of
metabolites in the ethyl acetate-soluble fraction and the Rf values of
metabolites in both the ethyl acetate-soluble and methanol-water-soluble
fractions differed from those in navy bean. The significant factor,
however, was that the percentage of $^{14}$C remaining as unmetabolized bentazon was 30.7% in black nightshade as compared to only 12.0% in navy bean. More metabolites were also found in cocklebur leaves than in the trifoliolate leaf of navy bean. As in black nightshade, numerous metabolites from cocklebur were also found to differ from those in navy bean as indicated by differences in Rf values and the greater quantity in the ethyl acetate-soluble fraction. Here again, the percentage of $^{14}$C remaining as unmetabolized bentazon in cocklebur was 61.4% as against 12.0% in navy bean.

From extensive investigations, Penner (218,219) and Mahoney and Penner (167,168) concluded that extensive diffusion of bentazon throughout the leaf, slow metabolism, continued total $^{14}$C accumulation in the leaf apex, and greater spray retention by the leaves of cocklebur all may have contributed to its observed susceptibility from foliar applications of bentazon.

L. Physical and Chemical Decomposition of Bentazon

Bentazon decomposes readily when exposed to high temperature (200 C) or ultraviolet radiation (270), or when stored in aqueous solutions for prolonged periods (36). This physical and chemical degradation of bentazon poses serious problems in studies dealing with the influence of $^{14}$C-herbicide on physiological and biochemical plant processes or uptake, translocation, and metabolism of $^{14}$C-herbicide. Some of the decomposition products of bentazon could enter the plant and interfere in the TLC separation and identification of $^{14}$C-metabolites in the plant. These decomposition products could have the same Rf value as plant metabolites. Such results would mislead an investigator if certain assumptions are made such as all the label entering the plant...
as parent herbicide molecule and a decomposition product as a plant metabolite.

Bentazon does not seem to persist as parent compound when stored in water. Bentazon was reported to be detected in large amounts in water only after hydrolysis with HCl. This suggests that much of the material is either conjugated in a form which could be broken down by HCl or that it simply needs acidification before it can be extracted in organic solvents. The major metabolite found in the water was N-isopropyl anthranilic acid amide, accounting for 27 to 34%, but bentazon was also present in water in significant amounts (66 to 73%) (36).

Eastin (82) reported that photolysis of bentazon gave at least 4 breakdown products separated by TLC. Bentazon was found to be degraded more by shortwave ultraviolet radiation than by longwave ultraviolet radiation or lights in growth chamber. Since bentazon is not volatile, Eastin (82) proposed that light may convert bentazon to a more volatile compound. But Dresher (78) did not see appreciable photodegradation of bentazon in solution that was irradiated through a 1 mm glass window for 20 h. This could be because of the inability of ultraviolet radiation to transmit through glass.

Nilles and Zabik (203) irradiated bentazon in water by sunlight for 115 h. They observed 8 degradation products totalling 36.2% which were not the products of hydrolysis. The major route of degradation was reported to be oxidative dimerization and nonconcerted loss of SO$_2$.

M. Fate of Bentazon in Plants

There is a general agreement in the most published reports on the nature of the metabolites of bentazon found in various plant species investigated. The first step seems to be the hydroxylation of bentazon
at the 6 or 8 position followed by conjugation with carbohydrates which makes these complexes more polar in nature. Otto and Dresher (213) extracted water-soluble $^{14}$C-metabolites of bentazon in soybeans at 50 and 20 days following foliar- and root-treatment, respectively. Otto (210) also observed an increase in the conjugation of label in treated soybeans up to 90 days.

Mahoney and Penner (167) isolated four methanol-water-soluble conjugates of bentazon from soybeans and navy beans which appeared to be similar although these authors could not determine their nature. Mahoney and Penner (168) also isolated more ethyl acetate-soluble and methanol-water-soluble metabolites from susceptible common cocklebur and black nightshade leaves than from tolerant soybeans or navy beans. All these compounds isolated by Mahoney and Penner (167,168) may not necessarily be the metabolic products of the plant. Some of them might as well be the photolysis and hydrolysis products of bentazon remaining on the leaf surface which appeared to be included in leaf extracts. Hayes and Wax (128) reported the formation of different polar metabolites of bentazon in treated soybean cultivars. The tolerant Clark 63 was found to contain metabolites I and II, whereas the sensitive PI 229.342 contained only metabolite II. Metabolite I was found labile to β-glycosidic activity while metabolite II remained unaffected. These results suggest that at least one of the metabolites in soybean is conjugated with glucose.

Kupelian (151) and Cannizzaro (45) reported aromatic hydroxylation of bentazon at 6 and 8 position followed by rapid conjugation of these materials into high molecular weight plant substances in soybeans. These results are supported by the findings of Otto et al. (211) who
also found the degradation of bentazon to proceed via hydroxylation of the aromatic ring at 6 and 8 position in soybeans. These metabolites were determined to be conjugated with carbohydrates, mono- and/or oligosaccharides. During maturation, these primary metabolites were found to degrade extensively. Since these hydroxylated bentazons are short lived, Otto et al. (211) could not isolate these intermediates of degradation from the soil. The glycosides obtained from soybean leaf extracts were cleaved by glycosidases liberating two aglycones which were less polar and similar to bentazon in TLC behavior. GC analysis revealed these compounds as 6-hydroxy- and 8-hydroxy-bentazon. In rice, however, only 6-hydroxy-bentazon was determined by GC analysis after cleavage of the conjugate (211).

Mine et al. (191) also reported that 85% of the absorbed bentazon was converted to a major water-soluble metabolite in tolerant rice, whereas in susceptible *Cyperus serotinus* only 25 to 50% was metabolized in 7 days. These authors identified the major metabolite in rice also as 6-(3-isopropyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide)-O-β-glucopyranoside. It appears that soybeans form two metabolites, 6- and 8-hydroxy-bentazon while rice metabolizes bentazon to only 6-hydroxy-bentazon.

Retzlaff and Hamm (233,234) reported that degradation or inactivation of bentazon to 6- and 8-hydroxylated compounds in wheat were catalyzed by hydroxylases. Both 6- and 8-hydroxy-bentazon from treated tolerant plants were demonstrated to be less toxic than bentazon (233).

Otto and Drescher (212) tried to specify the position on the ring to which the carbohydrates are attached. In studies with summer wheat, bentazon was shown to bind to the structural elements of the plant.
The one-third that is extractable consisted of soluble complexes (25%) as well as free bentazon (8%). They (212) suggested that the soluble complexes were probably formed in the plant as N-glycosides by the addition of mono- or poly-saccharides at the reactive 1-position of the bentazon molecule. In soybeans, 53% of the extractable residue consisted of unaltered bentazon; the remainder being in soluble complexes (214). Following the hydrolysis of the soluble complexes, Otto and Dresher (214) were able to detect not only the open ring hydrolysis product, N-2-carboxyphenyl-N'-isopropylsulfodiamide but also intact bentazon. They (214) reported that the metabolism of bentazon was qualitatively similar in wheat and soybeans. The metabolites of bentazon in spring wheat were also reported to be water-soluble conjugates, probably carbohydrate in nature, which also yielded bentazon upon hydrolysis (214). These reports show evidence that the conjugates are formed not only of the hydroxylated products of bentazon but also of intact bentazon.

Studies were also conducted to determine the channeling of the $^{14}$C-label from bentazon molecule into numerous biosynthetic processes and various natural plant constituents. Otto et al. (211) made labelled studies with $^{14}$C-bentazon to associate the fragments of bentazon with natural constituents of soybean and rice plants. They found that as the soybean plant matured, the methanol-extractable $^{14}$C-residues decreased and the presence of $^{14}$C-molecular fragments in lignin and lignocellulose increased. The structure of these bentazon fragments could not be elucidated as they were disintegrated by the usual drastic cleavage methods. In mature seeds, neither bentazon nor the conjugates of the two hydroxy bentazons were detectable, but 54% of the radioactivity was found to be located in the water soluble protein fractions, the soybean globulines.
The label in the protein was recovered in nearly all amino acids with maximum activity in aspartic acid, proline, glycine, alanine, and tryptophane. In rice, the conjugates of 6-hydroxy-bentazon were very rapidly degraded. At harvest, only traces of 6-hydroxy-bentazon were detectable in rice straw. The rice grains did not contain even traces of bentazon or its hydroxylated metabolite. In mature rice straw and grain, the label was found to be associated with or incorporated into high molecular constituents such as lignin, cellulose, starch, and protein. The radioactivity incorporated into lignin again could not be characterized in detail. But the label found in cellulose and starch were determined to be due to labelling of glucose. According to the scheme proposed by Otto et al. (211), the metabolism of bentazon results in fragments which enter the metabolic cycles of the plant and are used for the formation of natural plant constituents. These authors believe that 3-hydroxy anthranilic acid, a natural plant constituent in the tryptophane cycle, was the intermediate in the degradation of 8-hydroxy-bentazon. Although 8-hydroxy-bentazon was found only in small amounts, it probably was metabolized so rapidly that it could not be detected in large amounts. The evidence points to the stepwise degradation after cleavage of the aromatic ring resulting in simpler molecules which finally enter the citric acid cycle, the function of which could explain the association of the label in variety of plant constituents.

There are some disagreements in the reports, however, regarding the presence of bentazon residues in maturing plants. Most investigators reported that they could not detect any residues of bentazon in plants at harvest even at high rates of bentazon application (45,214). Foliar applications of $^{14}$C-bentazon to spring wheat indicated that there
was no movement of \textsuperscript{14}C out of the treated leaf. Because of its limited translocation, no \textsuperscript{14}C residues were detectable in the grain (214).

Eastin (81), however, reported that approximately 83\% of the label in the plant remained in shoot while half of it was found in mature panicle. At maturity, Eastin (81) found that 17\% of the extractable label to remain as bentazon all of which was located in the shoot. This disagreement in the results could be due to lack of proper identification techniques.

In several studies utilizing \textsuperscript{14}C-label, there are difficulties in accounting for the total label placed on the plant. Eastin (81) observed a decrease in \textsuperscript{14}C activity with time in treated rice plants. Eastin attributed this loss in activity to several factors he noted such as degradation to \textsuperscript{14}CO\textsubscript{2}, treated leaves falling off of the plant, and not harvesting all of the plant material.

N. **Effects of Bentazon on Respiration, Transpiration, and Photosynthesis**

Respiration, transpiration, and photosynthesis are the only physiological plant processes that are reported to be influenced by the application of bentazon. Penner (218,219) reported that foliarly-applied bentazon inhibited respiration both in tolerant soybeans and susceptible Canada thistle 1 day after the treatment; but only soybeans were found to recover from this inhibition 6 days later. This inhibition seems to occur only in the presence of light. Potter and Wergin (226) also observed a decrease in respiration in common cocklebur 2 h after bentazon application in light, but they found respiration to be unaffected in the dark.

McClelland et al. (177), however, reported an increase in the rates
of respiration due to bentazon application in susceptible morningglory species. These respiration rates were reported to decline on the fourth day. This observation was interpreted as an indication of dissipating bentazon activity and the plants resuming normal metabolic activity (178). But careful analysis of the experimental techniques performed by McClelland et al. (178) reveal that these authors measured CO$_2$ concentrations around the plants enclosed in a plastic bag to calculate photosynthetic-respiration equilibrium points (PRE-points). They attributed the observed initially declining PRE-points after bentazon treatment to increased respiration. The decreased PRE-points could, however, be due to decreased photosynthesis rather than increased respiration. Therefore, the reports of McClelland et al. (178) on increased respiration of bentazon-treated morningglories are questionable.

There are only two reports available on the influence of bentazon on transpiration. Penner (218,219) observed an increase in the transpiration rates of tolerant soybeans only for the first 24 h following the treatment. In susceptible Canada thistle, the transpiration rates were either slightly decreased or unaffected. How increased transpiration is related to tolerance of soybeans is not known.

Bentazon appears to have a more profound influence on photosynthesis than on any other plant process. Several workers reported an inhibition of photosynthesis in bentazon-treated plants as measured by decreased CO$_2$ uptake (26,63,175,189,190,218,219,225,226,233,234) or inhibition of Hill reaction (33,188,190,295). Some researchers specified the site of bentazon action as the inhibition of noncyclic electron transport at PS II without affecting PS I (33,220,234,253). The more recent work utilizing various artificial electron acceptors and donors proved that
bentazon inhibits electron transport at the site between the unknown primary electron acceptor for PS II (Q) and PQ complexes (253).

Photosynthesis in tolerant plants is either inhibited to a lesser extent than in susceptible plants (63, 218, 219) or the tolerant plants recover from this inhibition at a rapid rate by detoxifying bentazon (63, 189, 190, 219, 234).

A few workers tried to specify the mechanism by which bentazon causes irreversible inhibition of photosynthesis in susceptible plants. Böger et al. (33) found bentazon to inhibit noncyclic electron transport by isolated chloroplasts from the alga, *Bumilleriopsis filiformis* Vischer near PS II. They observed that the longer the incubation with bentazon, the more irreversible the inhibition even after transferring the chloroplasts to normal medium; which is unlike diuron [3-(3,4-di-chlorophenyl)-1,1-dimethylurea] and metribuzin where there is a quick and complete recovery upon transferring to normal medium (34). A slow partial recovery was possible if cells or isolated chloroplasts were exposed to bentazon for several days while the recovery was fast if exposed only for a few hours. These authors also demonstrated that the inhibition of electron transport was not due to deterioration of water-splitting reaction. Böger et al. (33) postulated that the irreversibility of long term inhibition of electron transport could be a metabolic process which brings about binding of bentazon or its active derivative to thylakoids in vitro but not in vivo. This would largely imply continued oxidation of NADPH which can ultimately lead to deficit of energy and oxidation of pigments like carotenoids and chlorophylls.

Potter and Wergin (226) gave support to the long-standing point of view that the observed inhibition of photosynthesis may be caused by
photo-induced toxic by-products due to misdirected channelling of light energy. They found that light was required for necrosis to develop in bentazon-treated leaves of cocklebur. The development of necrotic flecks was accelerated by increasing either the dose of bentazon or the level of illuminance. The time required to stop photosynthesis was independent of illuminance (21 to 86 Klux). However, photosynthesis was arrested more rapidly as the dosage of bentzon was increased from 0.05 to 1.0 kg/ha. Yet, this process always required at least 1 h irrespective of the rates used in these studies. The cessation of photosynthesis was found to precede cytological changes such as an abrupt degeneration of cell and chloroplast membranes in treated leaves. Regardless of the length of time required to stop photosynthesis, the time required for the development of necrosis after cessation of photosynthesis was directly related to the level of illuminance. Since the time delay between the cessation of photosynthesis and the development of toxicity symptoms increased as the level of illuminance decreased; this response led Potter and Wergin (226) to conclude that a toxic by-product is produced by misdirected channelling of light energy.

Although the findings of Potter and Wergin (226) do not answer all the pertinent questions, they do establish a basis for further investigations on why certain reactions are bentazon rate-dependent and certain others require specified amounts of time. Retzlaff and Hamm (233) worked further on results of Potter and Wergin (226) and presented a scheme by which the assimilation of CO$_2$ was influenced by varying amounts of bentazon in wheat plants. They found that maximum inhibition of assimilation always occurred at the same time after the treatment irrespective of the rates of bentazon used (0.25 to 16 kg/ha). The reduction in CO$_2$
assimilation rate was correlated with uptake of bentazon in leaf tissue. These results, in general, are not in disagreement with the previously published reports of Potter and Wergin (226).

Retzlaff and Hamm (233) suggested that treatment with bentazon initiates an enzyme adaptation which always requires the same length of time because of standard test conditions. After the enzyme has been formed, the uptake of $\text{CO}_2$ begins to rise again as metabolism of bentazon increases. The increase in inhibition of $\text{CO}_2$ assimilation with increasing rates of bentazon suggests that complete saturation of the receptor sites within the cells has not been reached in the range of amounts applied. The highest concentration of bentazon was reached in the leaf approximately at the same time the maximum inhibition of assimilation occurred. The concentration of bentazon then dropped; the rate of $\text{CO}_2$ assimilation and the amount of nontoxic 6-hydroxy-bentazon increased. They concluded that the concentration of hydroxylases which are necessary to metabolize bentazon to 6-hydroxy-bentazon and the rate of the reaction would determine whether herbicidal action occurs.

**0. A Possible Mechanism for Bentazon-Induced Phytotoxicity**

Early work with herbicides affecting the Hill reaction supported the assumption that these materials kill plants through starvation (175, 248). Mine and Matsunaka (190) showed that an exogeneous or endogenous supply of sucrose prevented bentazon injury to susceptible *Cyperus serotinus* Rottb. Further, the plants with large tubers were found to be more tolerant to bentazon than those with small tubers or no tubers. These investigators concluded that carbohydrates in tubers may have protected the plants from depletion of substrate caused by bentazon-inhibited photosynthesis. Retzlaff and Hamm (234) also explained that the
inhibition of CO\textsubscript{2} assimilation causes deficit of carbohydrates in weeds resulting in cessation of growth and finally followed by death.

Matsumaka (175) states that if the primary mode of action of a herbicide is the inhibition of photosynthesis, the treated plants are killed mainly by starvation, the development of herbicide injury occurs slowly, and the injury may be prevented or reversed by supplying plants with utilizable sugars. The idea that Hill reaction inhibitors kill the plants by starvation, however, was soon questioned by several other workers who found the lack of toxicity with these herbicides when the treated plants were not subjected to light (226). This prompted many investigators (14, 255) to propose that the toxicity was not caused by herbicide per se but rather by a secondary substance such as a toxic photosynthetic intermediate or a free radical formed by some mechanism involving the interaction between the herbicide and light. Ashton (12) later found that phytotoxicity of monuron [2-(p-chlorophenyl)-1,1-dimethylurea] and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-g-triazine] increased with increasing light intensity and also increased at the wavelengths of light absorbed by chl a and b. This eliminates the possibility of herbicidal toxicity due to starvation.

Krinsky (150) proposed a mechanism by which carotenoids function to inactivate an excited chlorophyll-oxygen complex which could be capable of catalyzing harmful photosynthesized oxidations in cells. Chlorophyll is excited by light and is usually deactivated to the original stable ground state by releasing its energy in the process of photosynthesis. If chlorophyll is not de-excited, it can combine with molecular oxygen and be destroyed by photooxidation. Some of the NADPH formed in the process of normal photosynthesis acts through an enzymatic
reaction to regenerate zeaxanthin, a carotenoid pigment, which can re-
duce the oxidized chlorophyll. In this process, NADPH is depleted which 
is necessary to restore carotenoids in reduced form. Therefore, this 
oxidation-reduction cycle can protect against lethal photo-oxidation of 
the chlorophyll molecules and other photosynthesized oxidations in the 
cell (116,150).

Bamji and Krinsky (22) have shown that malate can act as a reduc-
tant for oxidized carotenoids; and we can hypothesize that sucrose or 
other exogenously-supplied carbohydrate may have served as a precursor 
for malate and thus a reducing source for carotenoids and thereby 
allowing them to function in protecting chlorophyll from photo-oxidations 
and lowering toxicity symptoms. This might explain the results of Mine 
and Matsumaka (190) who reported prevention of bentazon injury by an 
exogenous supply of sucrose.

The time-course studies of Stanger and Appleby (248) showed that 
carotenoid pigments began to degrade before initiation of chlorophyll 
degradation due to phytotoxic actions of diuron on isolated chloroplasts 
of spinach. Similarly, bentazon might induce phytotoxicity by inhibit-
ing noncyclic electron flow (253) causing depletion of NADPH which is 
necessary to maintain carotenoids in reduced form (248) thereby prevent-
ing carotenoids from protecting chlorophyll photooxidations that lead to 
membrane disintegration which is visualized as necrotic flecks. When 
these necrotic flecks coalesce, the tissue would appear dead.

P. Pesticide Interactions

During the last two decades, the use of two or more pesticides on 
the same crop has become more prevalent in an attempt to broaden the 
spectrum of pests controlled. This practice often resulted in several
different interactions. These pesticide interactions may frequently occur from either simultaneous or sequential applications of two or more chemicals. Numerous interactions between herbicides, herbicides and insecticides, and herbicides and antidotes have been reviewed by Putnam and Fenner (230) many of which resulted in increased or decreased pest control. In several cases, these interactions also resulted in improved crop protection (54,55) or loss of selectivity of crop plants (90,283).

Tammes (262) classified four possible types of responses that may result from the combination of two phytotoxic chemicals: 1) an additive effect in which the injury is equal to the sum of the injuries produced by each of the components alone, 2) an independent effect in which the total injury is equal to the injury caused by the most active component alone, 3) an antagonistic effect in which the injury is less than the injury produced by the most active component alone, and 4) a synergistic effect in which the injury is greater than the sum of the injuries produced by the two components alone.

These pesticide interactions may occur in various ways. The pesticides may interact with each other in the spray tank (8) or on the plant surface (72) resulting in weakening or strengthening of physical binding with each other (8) or with the plant surface (72). Most of the interactions, however, generally occur at the plant surface or within the tissue influencing absorption or uptake (40,66,92,120,125,152,153,158,179,231,288), within the tissue influencing translocation (66,72,100,125,265), and metabolism (80,119,157,176,231,254,284,289), or by affecting critical physiological processes at the subcellular site of action (1,25,283).
I. **Herbicide-Herbicide Interactions**

The mechanism of action of many herbicide mixtures is not well understood (1). The present knowledge of the mode of action of individual herbicides in the mixture has not always explained the causes of observed synergism (1,91,283) or antagonism (9,25,76,157). Because, many herbicides are metabolically nonspecific, that is instead of a single site of action, there may be several sites and mechanisms through which phytotoxicity can be expressed (192). Although atrazine is primarily a photosynthetic inhibitor, Jordan et al. (142) showed inhibition of growth of cultured non-photosynthetic callus tissue of tobacco due to atrazine treatment which implicates that the mechanism of action is not limited to photosynthesis.

Baird et al. (19) reported that activity of glyphosate was reduced by mixing glyphosate with several other herbicides and this effect was overcome by increasing the rates of glyphosate. Appleby and Somabhi (8) demonstrated that the antagonistic activity of glyphosate on corn, beans, and quackgrass *Agropyron repens* (L.) Beauv. due to combinations with simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] or atrazine was due to physical binding of herbicides within the spray solution rather than from biological interactions within the plant. Arenstein (9), however, found that diuron reduced glyphosate activity even when applied 2 days before or after glyphosate application. Since much of the glyphosate enters the plant within 48 h after application (246), this would largely prevent any simple physical binding between the two herbicides on the plant surface. These results suggest that physical binding in the spray tank is not the only mechanism of antagonism between glyphosate and other herbicides. The other possibility that still remains to be
explored is that glyphosate might physically bind with the other herbicide within the plant tissue before the herbicides exert their lethal effects on active site.

Some of the herbicide interactions may be caused by increased or decreased uptake of one of the two chemicals. Eshel et al. (90) observed synergistic interactions from postemergence applications involving a carbamate herbicide, desmedipham [ethyl-m-hydroxycarbanilate carbanilate(ester)], and ethofumesate [(±)-2-ethoxy-2,3-dihydro-3, 3-dimethyl-5-benzofuranyl methanesulfonate] resulting in a wide spectrum of weeds controlled in sugarbeet fields. The young sugarbeet plants, however, were injured from this combination. From further investigations, Eshel et al. (92) found that ethofumesate increased the penetration of desmedipham into the foliage of three test species, redroot pigweed, wild mustard, and wild oats although there was no increase in the amount of desmedipham translocated out of the treated leaf (91). The basis for synergism, however, was not clear from their studies. Similar observations were also noted from the combinations involving other carbamate herbicides. McReynolds and Putnam (179) obtained synergism with the combinations of chlorpropham (isopropyl-m-chloro-carbanilate) and a urea herbicide, chloroxuron, applied to the foliage of onions, soybeans and white mustard (Brassica hirta Moench) plants due to enhanced foliar uptake of chloroxuron.

There are numerous reports on the interactions of triazines with other chemical groups which resulted in altered root uptake of one or more chemicals. Bucholtz and Lavy (40) studied the antagonistic interactions of alachlor and trifluralin with atrazine, cyanazine {2-[[4-chloro-6-(ethylamino)-a-triazin-2-yl]amino]-2-methylpropionitrile},
diuron, and methazole on oats. They found that alachlor and trifluralin caused inhibition of root growth which resulted in the decreased uptake of triazines. Similarly, trifluralin was found to protect soybeans from metribuzin or atrazine injury by reducing the uptake of these triazine herbicides through inhibition of soybean root development (153).

The suggestions on the mechanisms of interactions between triazines and thiocarbamates remain controversial. Duke et al. (80) reported an increase in EPTC (S-ethyl dipropylthiocarbamate) injury to alfalfa (Medicago sativa L.) grown in soils containing low levels of atrazine residues. They suggested that EPTC inhibits the formation of a detoxifying enzyme in alfalfa thus preventing atrazine metabolism. Wyse et al. (288), however, showed that EPTC increased the transpiration of navy bean by altering and reducing deposition of waxes on leaf surface. This increased transpiration was found to cause an increase in the uptake of atrazine from the soil. Leavitt and Penner (158) also observed an aggregation of the epicuticular wax layer of corn due to EPTC in an SEM study. EPTC, in these studies, was found not to block lipid synthesis in corn but was found to affect only wax arrangement on the leaf surface which resulted in increased cuticular transpiration of corn. This EPTC-induced wax aggregation was reported to result in predisposing corn to severe injury from subsequent postemergence applications of paraquat. Contrary to the results of Wyse et al. (288), atrazine itself was found to play a significant role in increasing transpiration by increasing stomatal openings of soybeans. Consequently, increased transpiration led to increased uptake of another triazine herbicide, metribuzin. This increase in metribuzin uptake was reported to cause synergistic inhibition of soybean growth (152). These observations suggest
that the interactions that resulted in increased or decreased uptake of
certain herbicides are probably species- and herbicide-specific.

Application of tank mixtures of methyl ester of diclofop {methyl
2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate}, a postemergence grass
herbicide, and 2,4-D [2,4-dichlorophenoxy]acetic acid] (95,209,265),
MCPA [(4-chloro-o-tolyloxy]acetic acid) (209,231), or dicamba (3,6-
dichloro-2-anisic acid) (209) has resulted in reduced control of wild
oats (231,265). Such interactions, however, were avoided by applying
the herbicides at different time intervals (207). Fletcher and Drexler
(95) failed to demonstrate the mechanism of antagonism in vitro using
excised leaf segments of cultivated oats. They suggested that changes
in uptake and translocation of methyl ester of diclofop might be in-
volved in intact plant which could not be demonstrated in leaf segments.
In their experiments, Fletcher and Drexler (95) used leaf segments from
mature leaves while the antagonism occurs only in meristematic tissues.
Other suggestions on the mechanism of antagonism between methyl ester of
diclofop and auxin-like herbicides include reduced uptake and metabolic
conversion of methyl ester of diclofop to the free acid, diclofop
{2-[4-(2,4-dichlorophenoxy)phenoxy]propanoic acid} (231) and reduced
translocation of diclofop to the site of action in meristematic tissue
(265).

The antagonistic interactions of methyl ester of diclofop are not
limited to auxin-like herbicides. Losses in annual grass control and
increased injury to soybeans were also reported with the combinations
of methyl ester of diclofop and bentazon. Such interactions were re-
duced by sequential application of the two herbicides (283). Dortenzio
and Norris (76) observed antagonistic interactions of methyl ester of
diclofop with desmedipham on barnyardgrass but not on littleseed canarygrass \( (Phalaris minor \text{ Retz.}) \). They could offset such antagonistic interactions on barnyardgrass by increasing the rates of methyl ester of dichlofop or delaying the application of desmedipham following the application of methyl ester of diclofop. These results led to the conclusion that antagonism of this herbicide mixture was species-dependent.

York and Slife (291) reported reduced corn injury when buthidazole \( [3-[5-(1,1\text{-dimethylethyl})-1,3,4\text{-thiadiazol-2-y1}]\text{-4-hydroxy-1-methyl-2imidazolidinone}] \) was applied to the soil in combination with alachlor or other acetanilide herbicides. This crop protection, however, was not seen when alachlor was applied to soil and buthidazole was applied post-emergence or when both herbicides were applied postemergence. Although, the mechanism of this interaction was not investigated, it appears that soil application of alachlor might reduce the uptake of buthidazole into corn roots as alachlor can be inhibitory to root growth of corn at 40% of the concentrations recommended for field applications (170). Alachlor does not inhibit shoot or root growth of corn due to foliar applications (170). This might explain the lack of interaction of alachlor with buthidazole in postemergence applications.

Ammonium thiocyanate was shown to cause increased translocation of amitrole (3-amino-s-triazole) in quackgrass indicating a synergistic effect of this mixture (72,100). The exact mechanism was not known, but Donnelley and Ries (72) suggested that ammonium thiocyanate lessened the strong binding of amitrole at the site of application whereas Forde (100) suggested that amitrole structure was altered to permit translocation.

Several workers reported antagonistic interactions between carbamate and auxin-like herbicides (24,139,227). Beste et al. (25) investigated
the causes of interactions between 2,4-D and EPTC. Addition of 2,4-D was found to be antagonistic to EPTC-induced inhibition of growth. Total RNA synthesis was increased by 2,4-D. EPTC alone, however, inhibited the synthesis of only r-RNA and 2,4-D has no effect on this selective inhibition. These results do not explain the relationship of RNA synthesis and causes of antagonism between 2,4-D and EPTC.

Akobundu et al. (1) observed a synergistic control of Japanese millet [Echinochloa frumentacea (Roxb.) Link] due to atrazine-alachlor combination. This synergism was attributed to synergistic inhibition of chloroplast protein synthesis. Woldetarios and Harvey (283) also noted a synergistic inhibition of soybean photosynthetic process due to methyl ester of diclofop and bentazon combination. The mechanism of this synergism is not known.

II. Herbicide-Antidote Interactions

Chemical antidotes are either applied to the soil directly or are used to coat crop seed to prevent herbicide injury to crop plants with little or no effect on the target weed species. Research in this area has been primarily concerned with the prevention of thiocarbamate herbicide injury to corn using various antidotes.

R-25788 (N,N-diallyl-2,2-dichloroacetamide) was found to protect corn from EPTC (49,54,158) and butylate (S-ethyl diisobutylthiocarbamate) (284) injury without loss of annual grass control. Earlier reports (93, 199) indicate that EPTC is detoxified in corn via hydrolysis. Recent studies (50,155,156), however, show that EPTC is detoxified by conjugation with glutathione. The increase of glutathione content mediated by stimulation of glutathione synthetase activity in corn due to R-25788 was later demonstrated by Carringer et al. (49). These findings suggest
that R-25788 accelerates EPTC detoxification via glutathione conjugation. Wright et al. (284) reported that R-25788 increased the metabolism of butylate in corn. They proposed that an alternate degradation pathway exists in corn due to protection by R-25788 but not in other grasses due to lack of this protection.

Leavitt and Penner (158) suggested a mechanism by which R-25788 protects corn from EPTC injury based on the morphological and structural changes on the leaf surface. They reported that EPTC caused an aggregation of the epicuticular wax layer in corn. This wax arrangement on leaf surface was found to predispose corn to injury from foliarly-applied paraquat. Soil application of R-25788 was reported to protect corn from paraquat uptake and injury by eliminating this EPTC-induced wax aggregation and preventing the predisposition effects on corn.

The increase in glutathione-S-transferase activity due to R-25788 seems to occur in response to only certain herbicides. Leavitt and Penner (157) did not see any protection to sorghum by R-25788 against injury caused by high rates of application of atrazine. The enzyme activity was not increased by R-25788. Therefore, they concluded that R-25788 may activate only thiocarbamate-specific enzymes but not atrazine-specific enzymes in sorghum and corn.

Another antidote, 1,8-naphthalic anhydride, was also reported to protect corn from butylate (63) and green foxtail [Setaria viridis (L.) Beauv.] from EPTC (54,55) injury. The exact mechanism of antagonism caused by this antidote is yet to be determined.

III. Herbicide-Insecticide Interactions

The interactions involving herbicides and insecticides have been shown to be both synergistic and antagonistic on crop and weed species.
Arle (10) reported that phorate [(0,0-diethyl S-[ethythio]-methyl]-phosphorodithioate] provided protection to cotton seedlings from the inhibitory effects of trifluralin on secondary root development. On the other hand, increased cotton injury resulted from the interaction of monuron, a urea herbicide, with carbamate insecticides (118,254) due to inhibition of a normal detoxifying mechanism in cotton (254).

Propanil was reported to cause toxicity to rice which had been treated with carbamate or phosphate insecticides (37,289). Matsunaka (176) found that these insecticides were involved in inhibiting an enzyme in rice which hydrolyzes propanil to form 3,4-dichloroacetanilide (289). This enzyme was partially purified and characterized as aryl acylaminidase (102).

The interactions involving chlorbromuron [3-(4-bromo-3-chlorophenyl)-1-methoxy-1-methylurea] (119) and alachlor (120) with carbofuran (2,2-dimethyl-2,3-dihydrobenzofuranyl-7-N-methylcarbamate) were reported to be synergistic in reducing the growth of barley. This effect was attributed to increased accumulation and decreased metabolism of chlorbromuron in shoots (119) and alachlor in roots (120) of barley.

The mechanism of pesticidal action is not always related to the observed synergism or antagonism. Furthermore, a pesticide does not always affect the same physiological process in two different species to cause interactions. In certain interactions, a particular pesticide may play a major role in killing the plant, while in others, the same pesticide might play only a secondary role by increasing the susceptibility of plant to the toxic effects of another chemical. The basis for interactions involving a particular pesticide, therefore, seems to vary with plant species and the other chemical it interacts with. Since the
mechanisms of interactions are probably species- and herbicide-specific, generalizations should not be made for the whole chemical class of pesticides or family of plant species based on individual observations.
September 17, 1981

Dr. S.R. Rao
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C.R. Swanson
Editor
Mefluidide-Bentazon Interactions on Soybeans (Glycine max) and Red Rice (Oryza sativa)

S. R. RAO and T. R. HARGER

ABSTRACT. Responses of soybeans (Glycine max (L.) Merr. 'Blaze') and red rice (Oryza sativa L.) to preemergence applications of mefluidide [2-(4,4-dimethyl-5-(1H,1H,3H,3H-methyl-2H-benzol[b]furan-3-yloxy)methyl)-2,2-diaza] and bentazon [1-(3-isopropyl-4H-1,3-benzo-thiazolin-2-yl)-3,3-dimethylurea] alone and in combinations were evaluated in greenhouse studies. Soybeans were tolerant to bentazon up to the highest tested rate of 2.0 kg/ha, but their height was reduced by mefluidide at 1.0 kg/ha. Soybeans were not injured by any tested combination of the two herbicides. Bentazon applied alone at rates of 0.5 kg/ha and above had a significant effect on red rice injury. Mefluidide applied at rates above 0.23 kg/ha severely reduced height and fresh weight of red rice. When these two herbicides were combined, a synergistic reaction occurred, and the combination was much more effective in controlling red rice than was either herbicide applied alone. The addition of a surfactant significantly increased the activity of the combination on red rice up to 0.23 kg/ha of mefluidide and at all rates of bentazon.

Additional index words. Herbicide combination, preemergence, synergism.

INTRODUCTION

Red rice is a weedy variety of cultivated rice and is considered to be the worst weed in rice fields of southwest Louisiana (5) and parts of the southern rice belt of the United States (19). Red rice presents a challenging control problem in the rice crop (1, 11, 19) because of the physiological and ecological similarities of the two varieties. Red rice grows taller than cultivated rice and lodges readily. This causes reduced grain yields (19). Red rice lowers the grade of milled rice (16) because of its objectionable dark-brown to red bran layer (1, 16). It shatters readily before harvest (19), thus establishing a population of dormant seed in the soil that can remain viable for 7 to 12 yr (B).

Soybeans are grown in rotation with rice as a means of reducing the red rice population in the soil (18, 19). Five percent or less escapees of red rice do not affect soybean production adversely, but can produce enough seed to restore original soil population levels (8). Therefore, complete control of red rice is more critical than with other weeds. The only effective means of controlling those plants that escape preplant incorporated and preemergence herbicides is by cultivation or directed postemergence applications of chloroturon [2-(p-(p-chlorophenoxy)phenyl]-1,1-dimethylurea] (18) and paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) (19). In the major rice production areas of southwest Louisiana, most farmers prefer to grow soybeans in a solid-seeded culture, which eliminates both cultivation and directed sprays. Diclofop [2-[4-(2,4-dichlorophenoxy)phenoxy)] propionic acid (1) was reported to give acceptable control of red rice in soybeans as an early postemergence treatment (4, 12). The results were inconsistent, however, and the control was unacceptable in the field experiments at various locations in Louisiana (5) and Arkansas (13). Mefluidide and bentazon applied in combination as an early postemergence treatment to soybeans gave more effective control of red rice (3, 9) and other problem weeds (5, 6, 9) than either herbicide applied alone (9). Slight crop injury (7, 10, 14, 17) and lodging of soybeans (10) was noted with mefluidide treatments of 0.5 kg/ha. Bentazon, however, was reported to cause only slight to no soybean injury at rates as high as 1.0 kg/ha (10, 17, 21).

Our objectives were to determine (a) the tolerance of soybeans to mefluidide and bentazon applied alone and in combination, (b) the response of red rice to different rates of mefluidide and bentazon applied alone and in combination, with and without a surfactant, and (c) the response of red rice to sequential applications of mefluidide and bentazon.

Volume 29, Issue 2 (March), 1981

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Table 1. Type of study, plant species, number of days from planting to treatment, stage of growth at treatment, and number of days from treatment to harvest (Experiments 1 through 4).

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Expt. (no.)</th>
<th>Species planted</th>
<th>Days from planting to treatment</th>
<th>Stage of growth at treatment</th>
<th>Days from treatment to harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean tolerance</td>
<td>1</td>
<td>Soybeans</td>
<td>12</td>
<td>30 to 40</td>
<td>19</td>
</tr>
<tr>
<td>Red rice control</td>
<td>2</td>
<td>Red rice</td>
<td>11</td>
<td>2 to 4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Red rice</td>
<td>14</td>
<td>3 to 6</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Red rice</td>
<td>11</td>
<td>3 to 6</td>
<td>21</td>
</tr>
</tbody>
</table>

*V* refers to completely unweeded trifoliolate leaf stage.

MATERIALS AND METHODS

**General.** Red rice seed was collected from rice fields in the vicinity of Crowley, Louisiana during the summers of 1977 through 1979. The seeds were air-dried at room temperature and stored at -1 C in sealed containers. The germination percentage varied from 96 to 88 during the course of investigation.

All tests were conducted in the greenhouse from August 1978 to June 1980. Either 20 seeds of soybeans or 40 seeds of red rice were planted 1 cm deep in a row in 25- by 15- by 7-cm styrofoam trays using unamended Olivier silt loam (6.1 pH, 1.4* CM) as the planting media. All the plants were germinated and grown under supplemental lighting provided by metal halide lamps as described by Duke et al. (2) from 6 a.m. to 8 p.m. The photosynthetic photon flux densities measured at plant height varied from 500 to 1,400 μmol* m⁻²* s⁻¹ on cloudy and sunny days, respectively. Temperatures and relative humidities were 40/25 ± 5 C and 50/90%, respectively, during day/night periods. The soil was wetted initially by subirrigation and later by daily overhead watering. The soil in all trays was supplemented with a commercial fertilizer containing 8% (w/w) each of N, P₂O₅, and K₂O at the biweekly rate of 25 kg/ha throughout the experiment. Soybean and red rice seedlings were thinned to 10 and 20 per tray, respectively, before herbicide application and all seedlings emerging subsequently were removed. Carbazyl (1-naphthyl N-methylcarbamate) was applied at 1 kg/ha in water periodically to control insects.

Herbicides were applied in water in 218 L/ha spray volume using a compressed-air, greenhouse belt sprayer with a flat-fan spray nozzle operated at 2.13 kg/cm² pressures. All treatments were replicated four times in a completely randomized design, and all experiments were repeated at least once over a period of time.

Data concerning time of application of postemergence treatments, size of plants in terms of height and leaf number at treatment, and time of harvest for all the experiments are summarized in Tables 1 and 2. All plants were harvested at the soil surface and fresh weight data of only the green shoots were recorded after taking plant height measurements. Plants were considered dead when no green tissue was visible, and dead tissue was discarded from partially killed plants before recording fresh weight data.

**Soybean tolerance.** Tolerance of soybeans to varying rates of mefluroidide, bentazon, and their combinations was evaluated (Experiment 1). Soybeans were at the first trifoliolate leaf stage at the time of treatment (Table 1). Herbicide treatments used are given in Table 3.

**Red rice control.** Three experiments were conducted to study the response of red rice seedlings at the 2- to 3-leaf stage to various rates of mefluroidide, bentazon, and their combinations. The growth of red rice at the time of treatment, however, was not the same in all three experiments (Table 1). A surfactant, alkyl aryl polyoxyethylene glycol*, was added at 0.25% (v/v) to all the herbicide treatments in Experiment 4.

**Herbicide timing intervals.** The experiment consisted of two sets of treatments (Table 2). In one set, a single application of mefluroidide was given 0, 2, 4, or 8 days after the application of bentazon. In the other set, the reverse sequence was followed, i.e., the application of bentazon was delayed by 2, 4, or 8 days following the application of mefluroidide. Mefluroidide and bentazon were applied at two rate combinations, 0.25 and 0.5 or 0.5 and 1.0 kg/ha, respectively. A surfactant* was added at 0.25% (v/v) to all the herbicide treatments.

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RAO AND HARGER: HERBICIDE INTERACTIONS

Table 3. Response of soybeans to methfluide and bentazon applied alone and in combination (Experiment 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (kg/ha)</th>
<th>Height (cm)</th>
<th>Fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methfluide</td>
<td>0.06</td>
<td>24.0</td>
<td>38.6</td>
</tr>
<tr>
<td>Methfluide</td>
<td>1.00</td>
<td>16.0</td>
<td>24.6</td>
</tr>
<tr>
<td>Bentazon</td>
<td>0.13</td>
<td>22.7</td>
<td>28.9</td>
</tr>
<tr>
<td>Bentazon</td>
<td>2.00</td>
<td>23.5</td>
<td>27.6</td>
</tr>
<tr>
<td>Methfluide + bentazon</td>
<td>0.06 + 0.13</td>
<td>21.8</td>
<td>26.7</td>
</tr>
<tr>
<td>Methfluide + bentazon</td>
<td>0.06 + 2.00</td>
<td>22.3</td>
<td>26.3</td>
</tr>
<tr>
<td>Methfluide + bentazon</td>
<td>1.00 + 0.13</td>
<td>22.0</td>
<td>28.4</td>
</tr>
<tr>
<td>Methfluide + bentazon</td>
<td>1.00 + 2.00</td>
<td>22.7</td>
<td>28.3</td>
</tr>
<tr>
<td>Uncontrolled control</td>
<td></td>
<td>2.4</td>
<td>NS*</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NS denotes nonsignificant at the 95% confidence level.

Treatments were started 10 days after planting and ended on day 18. The fresh weight data were recorded 3 weeks after the last day of treatment.

RESULTS AND DISCUSSION

Soybean tolerance. Soybean height was reduced when methfluide was applied at 1.0 kg/ha but the fresh weight was not reduced (Table 3). This height reduction was accompanied by leaf curling, which was apparent 1 week after the application of methfluide. Bentazon did not influence soybean growth at the highest rate of 2.0 kg/ha. No soybean injury occurred when methfluide was applied in combination with bentazon at 1.0 and 0.13 kg/ha, respectively. An interaction between these two herbicides appears to result in greater tolerance of soybeans to methfluide.

Red rice control. At the lowest rate tested (0.06 kg/ha), methfluide increased the height and fresh weight of red rice over the untreated control (Table 4). Similar observations were also noted by Trudove et al. (20) in corn (Zea mays L.).

Figure 1. Effect of bentazon and methfluide on the foliar fresh weight reduction of red rice expressed as percent of control. (a) No surfactant, (b) surfactant was added at 0.25% (Experiment 2). LSD’s (5%): herbicide rates = 5%, surfactant = 6%, methfluide X bentazon = 2%, methfluide or bentazon X surfactant = 7%, methfluide X bentazon X surfactant = 4%.

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At 0.25 kg/ha, mefluidide reduced fresh weight and height of red rice (Figure 1a). This growth reduction was accompanied by darkening of the leaves, increase in number of tillers, and callus formation near the basal node giving a "rosette" appearance to the plant. The leaves that emerged following the application of mefluidide failed to unroll. Bentazon alone had a significant effect on the growth of red rice only at rates of 5 kg/ha and above (Figure 2). At higher rates (>5 kg/ha), bentazon also caused necrosis of leaf tips. Rapid metabolism and detoxification is reported to be the selective mechanism involved in cultivated rice plants (15).

Combinations of bentazon and mefluidide were more effective in controlling red rice than either herbicide alone (Table 4, Figure 1a), indicating a synergistic effect. Additive (9) to synergistic (6, 9) control of hemp sesbania (Sesbania exaltata (Raf.) Cory) has been reported with this combination. As indicated by fresh weight reduction, the activity of the combinations was significantly increased up to 0.25 kg/ha of mefluidide due to addition of a surfactant (Figure 1). However, the activity of the mixture of the two herbicides was not improved when mefluidide was used at 0.5 kg/ha. Although high rates of both the herbicides were required for complete control of red rice under greenhouse conditions, our 2-yr field studies indicate that 0.25 kg/ha of mefluidide plus 0.5 kg/ha of bentazon with a surfactant at 0.25% (v/v) is sufficient to kill all red rice at the 3-leaf stage with no soybean injury. Synergistic interactions on red rice were also evident in these field studies. Treated red rice plants exhibited necrosis starting from leaf tip proceeding toward the base and from older to the younger leaves.

Addition of a very low amount (0.13 kg/ha) of bentazon to a high rate of mefluidide (1 kg/ha) did not reduce height or fresh weight more than that reduced by mefluidide (1 kg/ha) alone (Table 4). On the other hand, bentazon alone at a high rate (2 kg/ha) did not influence red rice growth, but addition of a very small amount (0.06 kg/ha) of mefluidide reduced plant height and fresh weight significantly.

Herbicide timing interval. Fresh weight reduction was greatest when both the herbicides were applied together as a mixture (Figure 3). At both rates of herbicide application, delaying the application of mefluidide by 8 days following the application of bentazon caused significantly more fresh weight reduction than following the reverse sequence. This effect was not measurable after a 3- or 4-day delay.

These studies establish that it is safe to use the combinations of mefluidide and bentazon at rates that are toxic to red rice as over-the-top postemergence treatments on soybeans. Addition of bentazon to mefluidide reduced soybean foliage crinkling caused by the application of mefluidide alone.

Either mefluidide at 1.0 kg/ha or bentazon up to 30 kg/ha alone failed to give acceptable control of red rice, but the combination of the two herbicides was synergistic and provided good control of red rice when the seedlings were at the 2- to 3-leaf stage. The combination of the two herbicides plus a surfactant not only reduces the required rates, but also reduces the risks of soybean injury.

When delayed applications of one component of the combinations on red rice is considered (Figure 3), the residual herbicidal effect of mefluidide disappears more rapidly than that of bentazon. Additional research is warranted with the
combination of these two herbicides at low rates with a surfactant under varying field conditions to determine the extent of damage, if any, to soybeans and any possible loss of activity on other weed species. Further studies are in progress to elucidate the cause of the observed synergistic interactions of methidathion and bentazon on red rice.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the partial financial support by the Louisiana Soybean Promotion Board, BASF Corp., and 3M Company. The authors also wish to thank F. R. Nester and P. S. Johnsey for technical assistance.

LITERATURE CITED

The following two manuscripts will be presented in their present form to the Weed Science Society of America for publication in Weed Science.
Mechanism of Interaction Between Mefluidide and Bentazon on Red Rice (Oryza sativa). I. Loss, Uptake, and Translocation of the Herbicides

SUDABATHULA RAO and T.R. HARGER

Abstract. Loss, uptake, and translocation of foliarly-applied mefluidide \( N-[2,4\text{-dimethyl-5-}[[\text{trifluoromethyl}}\text{ sulfonyl}] \text{amino}] \text{phenyl} \) acetamide and bentazon \( [3\text{-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)}\text{-one 2,2-dioxide}] \) alone or in combination with each other to red rice \( [Oryza sativa L. 'Strawhulled'] \) were compared. When applied alone, bentazon did not affect red rice while mefluidide only reduced the height. Combinations of these two herbicides resulted in synergistic interactions causing severe necrosis and death of red rice within 6 days after treatment (DAT). Loss of radioactivity occurred from both living and dead red rice plants treated with \(^{14}\text{C}-\text{bentazon} or \(^{14}\text{C}-\text{mefluidide} when applied alone or in combination with the other non-radioactive herbicide until the termination of the experiment at 6 DAT. Combination with bentazon reduced the loss of \(^{14}\text{C}-\text{mefluidide} from living plants from 38.4 to 31.5\% at 6 DAT but did not affect loss from dead plants.}

Received for publication

This is a portion of the senior author's dissertation for the Ph.D. degree.


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The addition of mefluidide reduced the loss of $^{14}$C-bentazon from 45.4 to 23.4% from dead plants but did not affect loss from living plants. The maximum amount of radioactivity from both of the herbicides in single and combination treatments was found inside the plant at 2 DAT with a severe decline at 4 and 6 DAT only in combination treatments. The herbicide combinations resulted in less uptake of both mefluidide and bentazon than when either was used alone. The translocation of both herbicides from the treated-middle leaf was predominantly acropetal with limited basipetal movement after five days. Treating the entire plant with bentazon significantly reduced the translocation of radioactivity from $^{14}$C-mefluidide applied to only the middle leaf; however the entire plant showed necrosis. Mefluidide sprayed on entire plant significantly increased the translocation of radioactivity from $^{14}$C-bentazon applied to the middle leaf only, but visual symptoms of injury were observed only on the bentazon-treated leaf. The changes in loss, uptake, and translocation of $^{14}$C-mefluidide due to addition of bentazon and of $^{14}$C-bentazon due to addition of mefluidide were significant, however, they could not account fully for the observed synergistic interactions of the mefluidide-bentazon combinations on red rice.

Additional index words. Volatilization, degradation, absorption, penetration, synergism, herbicide combinations.

INTRODUCTION

During the last two decades, the use of two or more herbicides on the same crop has become more prevalent in an attempt to broaden the spectrum of weeds controlled. This practice has often resulted in several different interactions and the responses are categorized as
synergistic, antagonistic, additive, or independent (29). These herbicide interactions may occur from the use of two or more chemicals simultaneously or sequentially. Numerous interactions between herbicides have been recently reviewed by Putnam and Penner (26). Interactions have not only resulted in increased (13, 22) or decreased (1, 34) weed control but also in increased crop tolerance (6, 7) or loss of selectivity of crop plants (12, 34).

Several workers reported synergistic control of red rice in soybeans [Glycine max (L.) Merr.] with the postemergence applications of combinations of mefluidide and bentazon (2, 11, 28). Mefluidide, applied alone, caused height reduction whereas bentazon alone had negligible activity on red rice (28). The synergism of foliarly-applied herbicide combinations could be a function of changes in herbicide losses from the plant (17), amount of herbicide retained (20, 25), absorption or uptake (14, 21, 35), translocation within the plant (21, 30, 31), metabolism of one or more chemicals (19, 20, 23, 27), or critical physiological plant processes at the subcellular site of action (3, 34), among other factors.

Uptake, translocation, and metabolism of mefluidide (4, 5) and bentazon (20, 25) on selected plant species have been previously reported. However, the basis for the interaction of mefluidide and bentazon either on red rice or on other weeds has not been clearly established. Eastin (10), from his autoradiograms, observed an increase in the translocation of radioactivity from 14C-bentazon from the treated leaf over time when in combination with mefluidide. He concluded from his studies that the mechanism of synergism of this herbicide mixture on red rice may be due to increased movement of bentazon.
within red rice plants to the site or sites of phytotoxic action. However, it is not clear whether the amount of $^{14}$C translocated out of the treated leaf was sufficient to account for the observed synergism and if the translocated $^{14}$C was bentazon or its metabolite(s).

In several studies utilizing $^{14}$C, there is difficulty in accounting for the total radioactivity applied to the plant (8, 9). Eastin (9) observed a decrease in total radioactivity with time in $^{14}$C-bentazon treated rice (Oryza sativa L.) plants. He attributed this loss to degradation to $^{14}$CO$_2$, among other factors. The studies of Glenn and Rieck (16) also indicate that 7% of the total $^{14}$C-mefluidide applied to corn could not be accounted for.

The objectives of this investigation were to evaluate differences in loss, uptake, and translocation of mefluidide and bentazon when applied alone and in combination on red rice as possible mechanisms for synergistic interactions.

**MATERIALS AND METHODS**

**General.** Strawhulled red rice seed was collected from soybean fields at Burden Research Plantation, Baton Rouge, Louisiana during the summer of 1979. The seed was air-dried at room temperature and stored at -1 C in sealed containers until use. The germination percentage varied from 95 to 90 during the course of these studies.

All plants were germinated and grown in a greenhouse. Ten seeds were planted 1 cm deep in 16-cm-diameter styrofoam pots using a mixture of Olivier silt loam soil (6.1 pH, 1.4% organic matter) and sand (1:1, w/w) as potting medium. Natural light was supplemented with metalhalide lighting (500 to 1400 $\mu$Em$^{-2}$s$^{-1}$) from 6 a.m. to 8 p.m. Tem-
Temperatures and relative humidities were 35/24 ± 5°C and 50/90%, respectively, during day/night periods. All plants were surface watered once daily until herbicide application, after which plants were only subirrigated. The potting medium was supplemented once with a commercial fertilizer containing 8% (w/w) each of N, P₂O₅, and K₂O at the rate of 25 kg/ha 7 days after planting. Red rice seedlings were thinned to one per pot before herbicide application and all seedlings emerging subsequently were removed.

Over-the-top applications of commercial formulations of herbicides were made in water in 200 L/ha spray volume containing 0.25% (v/v) of a surfactant, alkyl aryl polyoxyethylene glycol³ using a compressed-air greenhouse moving belt sprayer with a flat-fan spray nozzle at a pressure of 1.83 kg/cm². The working ¹⁴C-solutions were prepared by formulating technical ¹⁴C-bentazon (phenyl-U-¹⁴C, specific activity 13.7 mCi/mM)⁴ as a sodium salt by reacting with NaOH and technical ¹⁴C-mefluidide (phenyl-U-¹⁴C, specific activity 12.9 mCi/mM)⁵ as a diethanol amine salt using dilute solutions of diethanol amine (pH 8.0) each containing 0.25% (v/v) of the surfactant³. The working ¹⁴C-solutions of bentazon and mefluidide contained 1 µCi in 14.9 and 16.9 µl water, respectively. In all experiments, the total herbicide dose was adjusted to field use rates of 0.3 and 0.6 kg/ha for mefluidide and bentazon, respectively. The amount of herbicide a red rice plant would receive was estimated by measuring

³Triton Ag-98.
⁴BASF Corp.
⁵3M Company.
the average leaf area that would intercept the herbicide spray utilizing polaroid pictures taken from directly above the plants at 3 leaf-stage. Based on this leaf area, the rates of herbicides applied as over-the-top sprays were adjusted to supplement the radioactive herbicide and bring the total dose to the desired field use rate.

In all experiments, treatments were replicated four times in a completely randomized design and all experiments were repeated once. All percentage data were subjected to arc sin transformations before statistical analysis (ANOV). The mean comparisons of percentage data presented in the tables are applicable only to the transformed data. Protected Fisher's least significant difference was used for mean separation.

Herbicide loss. The loss of mefluidide and bentazon applied alone or in combination from living and dead red rice plants was determined by measuring the total radioactivity recovered after various time periods. The dead plants were obtained by removing the plants from potting medium and allowing them to dessicate to death for 4 days. The dessicated plants were returned to potting medium for support during the course of the experiment. Fifteen μl of working solution of 14C-bentazon (0.15 kg/ha) or 17 μl of working solution of 14C-mefluidide (0.2 kg/ha) was applied as 100 drops randomly to the adaxial surface of all the leaves of 3-leaf (20 to 22 cm, 13 day-old) living and dead red rice plants using a micro-syringe. Just prior to the placement of the radioactive herbicides, red rice plants were supplemented with over-the-top sprays of commercial formulations of mefluidide, bentazon, or mefluidide plus bentazon on greenhouse belt sprayer. The treatments are given in Table 1.
For analysis, the plants were removed from the potting medium with as much root system intact as possible at 0, 2, 4 or 6 DAT. The root system was washed with 10 ml water directed on the roots from a wash bottle to remove the potting media. The whole plant was combusted in oxygen with a biological oxidizer (Harvey BMO) and $^{14}$CO$_2$ was trapped in 15 ml of commercially prepared scintillation cocktail, Scintisorb-C (Isolab). The radioactivity in the samples was determined by liquid scintillation spectrometry (LSS) (Beckman LS-250). Soil samples were also taken from each pot and analyzed for radioactivity. Each 50-g-soil sample was homogenized (Brinkmann, Polytron PCU-2-110) three times each for 1 min with 100 ml of either acetone or methanol for bentazon or mefluidide samples, respectively. The extracts were pooled, vacuum filtered (Whatman 42), and condensed to 10 ml on a rotary evaporator (Büchi Rotavapor-R) at 55 C. A 1-ml aliquot was added to 15 ml of commercially prepared scintillation cocktail, Scintisol (Isolab). The radioactivity in the samples was quantified by LSS. All samples were corrected for quenching using a standard curve from channel ratio method and background radiation. The radioactivity not recovered was expressed as percent lost based on total $^{14}$C applied.

**Uptake study.** Red rice plants in the 3 leaf-stage (20-22 cm) were treated with commercial formulations and working solutions of $^{14}$C-herbicides the same way as described in herbicide loss study. The plants were removed from the potting medium at various DAT as described in previous study after making visual observations and height measurements. The root system was rinsed with 10 ml water as described before and the plants were separated into shoot and root. The entire shoot was washed in 100 ml water in a graduated cylinder for 10 min with agitation.
and then rinsed twice each with 10 ml water and once with 10 ml acetone from wash bottle to remove any unabsorbed chemicals on the plant surface. All washes from a single plant were pooled, vacuum filtered (Whatman 42), acetone was evaporated on a rotary evaporator at 55 C, all solutions were adjusted to equal volumes (130 ml) with water, and a 1-ml aliquot was assayed by LSS. The plant washes were saved for further analysis. This washing technique was found to recover 96 to 98% of these herbicides from the plant surface without extracting plant pigment as measured by absorption spectrophotometry (Perkin-Elmer 124). The washed shoots were extracted by homogenizing three times each with 50 ml absolute methanol. The first extraction was for 1 min, and the later two for 0.5 min each. The extracts were pooled, vacuum filtered (Whatman 42), all solutions were adjusted to equal volumes (160 ml) with methanol, and 1-ml aliquot was assayed by LSS. The methanol shoot extracts were saved for further analysis. The unextractable shoot residues, residues from filtering the plant washes, and roots were separately combusted in an oxidizer and the radioactivity in the samples were quantified by LSS as described earlier in herbicide loss study. The radioactivity in the soil samples was assayed as described in herbicide loss study. The uptake was determined as the sum of $^{14}$C recovered in methanol extract, unextractable plant residue, residue from filtering the surface wash, and root expressed as percentage of total dpm applied to the plant. The unaccountable radioactivity was assumed to be losses from degradation and/or volatilization from the plant.

**Translocation study.** Red rice at the 3 leaf-stage (14-16 cm, 11 day-old) was sprayed with commercial formulations of mefluidide (0.3 kg/ha) or bentazon (0.6 kg/ha) on a table sprayer. Immediately fol-
lowing, 2 μl of working solution of ^14C-mefluidide (0.12 μCi) or 5 μl of working solution of ^14C-bentazon (0.34 μCi) was placed as 0.5 μl drops in a 1 cm² area toward the middle of adaxial surface of the middle leaf. This amount was equivalent to a field rate of application of 0.3 kg/ha for mefluidide and 0.6 kg/ha for bentazon on the 1 cm² area. Visual observations and height measurements were made and plants were removed from the soil 5 DAT with as much intact root system as possible and separated into roots and individual leaves. These samples were combusted separately and ^14C was quantified as described before. Soil samples were also analyzed for radioactivity as described earlier. The treatments are given in Table 3. Data were expressed as percentage of radioactivity placed on the plant.

RESULTS AND DISCUSSION

Herbicide loss. When the herbicides were used in combination, visual symptoms of injury appeared on living plants one DAT in the form of necrotic flecks over the entire plant. The necrotic flecks started to coalesce near the tip of the leaf and proceeded toward the base of the plant covering the entire plant by 4 DAT. The plants appeared dead at 6-day sampling. When used alone, bentazone did not affect the growth of red rice; whereas mefluidide alone caused height reduction and callus formation near the base of the plant but did not cause necrosis.

There was a continuous loss of both ^14C-mefluidide and ^14C-bentazon from living as well as dead red rice plants with time until the experiment was terminated at 6 DAT (Table 1). The loss of ^14C-mefluidide, when used alone or in combination with bentazon, was significantly greater from living plants as compared to dead plants. The addition of
Table 1. The loss of radioactivity from $^{14}$C-mefluidide and $^{14}$C-bentazon from living and dead red rice plants at 0, 2, 4, and 6 DAT.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Loss of radioactivity at various DAT&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Living plant</td>
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<tr>
<td>$^{14}$C-mefluidide</td>
<td>0.6</td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>0.5</td>
</tr>
<tr>
<td>LSD 0.05</td>
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<tr>
<td>$^{14}$C-bentazon</td>
<td>0.3</td>
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<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>0.7</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td></td>
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</tbody>
</table>

<sup>a</sup>Losses were expressed as percentage of the total radioactivity placed on the plant.
bentazon significantly reduced the loss of $^{14}$C-mefluidide from living plants but did not affect the loss from dead plants. These results suggest that the loss of mefluidide from living plants is not solely due to physical forces but may be due also to physiological plant processes which degrade mefluidide to $^{14}$CO$_2$ or volatilize its metabolites. The addition of bentazon caused disruption of plant metabolism as evidenced by necrotic tissues one day after treatment. This disruption of plant metabolism probably contributed to the decreased loss of mefluidide from living plants.

The loss of $^{14}$C-bentazon, when used alone, was greater from dead plants than living plants. The addition of mefluidide, however, reduced the loss of $^{14}$C-bentazon from dead plants but not from living plants. These results indicate that there may be a binding between bentazon and mefluidide or an additive in the mefluidide formulation which resulted in reduced volatilization of $^{14}$C-bentazon from dead plants. The rapid metabolism and conjugation of bentazon, when used alone, by tolerant rice plants may explain the reduced loss (23). The metabolism of bentazon would be reduced because of the disruption of metabolic processes due to combination with mefluidide.

The herbicide loss study, however, can not clearly establish if the loss of these herbicides was from plant surfaces or inside the plants. Samples of potting medium were found to contain no detectable radioactivity. Since the visual symptoms (28) of injury on living plants in the combination treatments were first noticeable a day after treatment, the small differences in the losses of radioactive herbicides from the treated plants at 2 DAT probably contributed little to the observed synergism.

Uptake study. The visual symptoms of injury were evident at one
DAT and the plants appeared dead at 6 DAT when the herbicides were used in combination as described in previous study with the living plants. Bentazon, when applied alone, did not affect red rice while mefluidide alone caused height reduction (Table 2) and callus formation near the base of the plants.

The uptake of radioactivity from $^{14}$C-mefluidide when used alone or in combination with bentazon and from $^{14}$C-bentazon when used alone or in combination with mefluidide reached their maximal level approximately 2 DAT (Table 2). There was a decline, however, in the amount of $^{14}$C inside the treated plants at 4 and 6 DAT. This decline was found not due to exudation of herbicides or their metabolites into the potting medium through the root system as no $^{14}$C was present in the potting medium in detectable amounts. Care was also exercised in harvesting the entire plant including the dead leaves and detached roots in order to account for the total amount of $^{14}$C placed on the plant. This decline could, therefore, be due to losses of the herbicides or their metabolites as $^{14}$CO$_2$ into the atmosphere from inside the plant due to metabolism still proceeding in the green portions of the plant. Such degradation losses of $^{14}$C-bentazon to $^{14}$CO$_2$ in rice were suggested by Eastin (9). The loss could also be the result of volatilization, microbial degradation, or leaching from dead portions of red rice.

There were no significant differences in the amount of $^{14}$C-mefluidide lost from inside the plant when it was used alone or in combination with bentazon, but the amount of $^{14}$C-bentazon lost from inside the plant was significantly higher when it was used in combination with mefluidide than when used alone. These results suggest that the loss of these herbicides from a living plant reported in the previous study (Table 1)
Table 2. Uptake of radioactivity from $^{14}$C-mefluidide and $^{14}$C-bentazon by red rice plants at 0, 2, 4, and 6 DAT.

<p>| Treatment       | Visual ratings $^a$ | Recovery of radioactivity $^b$ |  |  |  |  |  |  |
|-----------------|---------------------|--------------------------------|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th></th>
<th>(%)</th>
<th>Shoot</th>
<th>Root</th>
<th>Total Foliage</th>
<th>Extract</th>
<th>Residue</th>
<th>uptake</th>
<th>wash</th>
<th>(%)</th>
</tr>
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<tbody>
<tr>
<td>At 0 DAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>0 21</td>
<td>1.6</td>
<td>0.1</td>
<td>0.0</td>
<td>1.7</td>
<td>99.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>0 22</td>
<td>1.7</td>
<td>0.1</td>
<td>0.0</td>
<td>1.8</td>
<td>99.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>0 20</td>
<td>1.4</td>
<td>0.5</td>
<td>0.0</td>
<td>1.9</td>
<td>98.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>0 22</td>
<td>2.4</td>
<td>0.2</td>
<td>0.0</td>
<td>2.6</td>
<td>97.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 2 DAT</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>$^{14}$C-mefluidide</td>
<td>20 21</td>
<td>42.1</td>
<td>12.1</td>
<td>0.3</td>
<td>54.5</td>
<td>26.1</td>
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<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>78 19</td>
<td>39.6</td>
<td>9.9</td>
<td>&lt;0.1</td>
<td>49.5</td>
<td>35.9</td>
<td></td>
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<tr>
<td>$^{14}$C-bentazon</td>
<td>5 22</td>
<td>56.6</td>
<td>12.8</td>
<td>0.1</td>
<td>69.5</td>
<td>17.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>78 18</td>
<td>37.1</td>
<td>22.0</td>
<td>0.1</td>
<td>59.2</td>
<td>29.1</td>
<td></td>
<td></td>
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<tr>
<td>At 4 DAT</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>$^{14}$C-mefluidide</td>
<td>28 18</td>
<td>38.6</td>
<td>12.6</td>
<td>0.6</td>
<td>51.8</td>
<td>16.0</td>
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<td>$^{14}$C-mefluidide + bentazon</td>
<td>88 9</td>
<td>31.1</td>
<td>12.8</td>
<td>&lt;0.1</td>
<td>43.9</td>
<td>34.0</td>
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<tr>
<td>$^{14}$C-bentazon</td>
<td>3 24</td>
<td>51.7</td>
<td>16.9</td>
<td>0.2</td>
<td>68.8</td>
<td>13.3</td>
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(continued)
### Table 2. Continued.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visual ratings&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Height (cm)</th>
<th>Recovery of radioactivity&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shoot Extract</td>
</tr>
<tr>
<td>14C-bentazon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ mefluidide</td>
<td>86</td>
<td>10</td>
<td>23.8</td>
</tr>
<tr>
<td>At 6 DAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14C-mefluidide</td>
<td>50</td>
<td>16</td>
<td>34.2</td>
</tr>
<tr>
<td>14C-mefluidide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ bentazon</td>
<td>99</td>
<td>2</td>
<td>28.4</td>
</tr>
<tr>
<td>14C-bentazon</td>
<td>0</td>
<td>26</td>
<td>48.0</td>
</tr>
<tr>
<td>14C-bentazon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ mefluidide</td>
<td>100</td>
<td>0</td>
<td>20.7</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>9</td>
<td>4</td>
<td>3.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control was estimated visually (0 = no effect and 100 = complete kill).

<sup>b</sup> Data were expressed as percentage of the total radioactivity placed on the plant.
are not only from the plant surfaces but also from inside the plant. This could be expected because in the combination treatments, the necrosis of the leaves increased during the course of the experiment which could have resulted in more radioactivity being removed from inside the leaf with the washing procedure.

There was significantly less uptake of $^{14}$C-mefluidide as compared to $^{14}$C-bentazon by treated red rice. The addition of bentazon to $^{14}$C-mefluidide and mefluidide to $^{14}$C-bentazon caused significantly less uptake of both $^{14}$C-herbicides by red rice. The localized injury on the treated areas caused by the combination treatments might have impeded the uptake process of both the herbicides. Since the reduced loss of $^{14}$C-mefluidide from living plants observed in the combination treatment in the previous study (Table 1) did not contribute to its increased uptake, the loss of $^{14}$C from $^{14}$C-mefluidide appear to be equally contributed both from inside and surface of living plants (Tables 1 and 2).

Most of the $^{14}$C from bentazon and mefluidide absorbed inside the plant remained in the shoot with very little translocated to the root. There was an increase in the amount of $^{14}$C in unextractable residue with time in all the treatments. The $^{14}$C in the extract, however, increased up to 2 DAT and then declined at 4 and 6 DAT. There was more radioactivity from $^{14}$C-bentazon in the unextractable residue when it was applied in combination with mefluidide than when it was applied alone. The high amounts of $^{14}$C in the residue in combination treatments could be due to incorporation of the $^{14}$C into cell wall constituents and other unextractable plant tissues due to continued metabolism in the green portions of the tissue. Otto et al. (24) observed an increase with time in the amount of $^{14}$C from bentazon to be associated with high
molecular constituents such as lignin, cellulose, starch, and protein in cultivated rice. The addition of mefluidide may have caused changes in the metabolism of bentazon contributing to increased incorporation of radioactivity from bentazon in these plant constituents. The amount of radioactivity from $^{14}C$-mefluidide in the unextractable residue, however, did not differ between $^{14}C$-mefluidide and $^{14}C$-mefluidide plus bentazon treatments. There is more $^{14}C$ from $^{14}C$-mefluidide translocated into the root when $^{14}C$-mefluidide was used alone than when combined with bentazon. The poor translocation of $^{14}C$ to the root may not necessarily be a result of less uptake of $^{14}C$-mefluidide but of impaired transport caused by severe necrosis of the treated shoot in the combination treatment. There was no significant difference between $^{14}C$-bentazon and $^{14}C$-bentazon plus mefluidide treatments in the translocation of $^{14}C$ to the root. Because there is less total radioactivity inside the plant at 2 DAT from both herbicides when applied in combination than when applied alone, the amount of absorption does not explain the observed synergism.

**Translocation study.** When a small area on the middle-leaf of a red rice plant was treated with either $^{14}C$-mefluidide or $^{14}C$-bentazon, there were no visual symptoms of injury even on the treated area and the plants grew normal to the fourth-leaf stage (Table 3). When $^{14}C$-mefluidide was placed on the middle-leaf and the entire plant was sprayed with bentazon, the whole plant was retarded in its growth at 3-leaf stage and necrotic flecks appeared on the entire plant. When $^{14}C$-bentazon was placed on the middle-leaf and the entire plant was sprayed with mefluidide, the plants failed to develop the fourth-leaf and showed necrotic flecks only on the treated-leaf without affecting the rest of the plant.

The amount of total $^{14}C$ recovered was greater from $^{14}C$-mefluidide
Table 3. Translocation of radioactivity from $^{14}$C-mefluidide and $^{14}$C-bentazon by red rice plants at 5 DAT.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visual ratings$^a$ (%)</th>
<th>Height (cm)</th>
<th>Distribution of $^{14}$C in various plant parts$^b,c$ (%)</th>
<th>Total $^{14}$C recovered$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>10</td>
<td>17</td>
<td>0.58 3.40 67.34 5.45 1.23</td>
<td>78.00</td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>58</td>
<td>6</td>
<td>0.19 1.85 71.64 5.88  ---</td>
<td>79.59</td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>0</td>
<td>19</td>
<td>0.33 0.17 66.23 0.80 0.20</td>
<td>67.77</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>24</td>
<td>16</td>
<td>0.30 0.97 63.32 2.42  ---</td>
<td>67.02</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>12</td>
<td>0.8</td>
<td>Plant part x chemical treatment = 0.45</td>
<td>1.70</td>
</tr>
</tbody>
</table>

$^a$Control was estimated visually (0 = no effect and 100 = complete kill).

$^b$Data were expressed as percentage of the total radioactivity placed on the plant.

$^c$L1 to L4 refer to oldest to youngest leaves.

$^d$L2 was the treated leaf.
treated plants as compared to those treated with $^{14}\text{C}$-bentazon. There was no influence of one chemical on the other on the amount of total $^{14}\text{C}$-recovered in this study. There is a discrepancy in the total amount of $^{14}\text{C}$ recovered in combination treatments between the translocation study (Table 3) and the herbicide uptake study (Table 2). This disagreement could be because of the localized placement of the radioactivity in the translocation study as compared to treating the entire plant with the radioactivity in the uptake study. The losses due to leaching are probably minimal as no radioactivity was present in potting medium.

The data on the distribution of $^{14}\text{C}$ in various plant parts show that the translocation of $^{14}\text{C}$ from both herbicides used alone or in combinations was primarily acropetal with limited basipetal movement. These results are in agreement with the previously published reports which state that both mefluidide (4, 15, 33) and bentazon (18, 19, 23, 32) move primarily in acropetal direction with limited basipetal translocation.

Bentazon, when sprayed over plants treated with $^{14}\text{C}$-mefluidide, reduced the total amount of $^{14}\text{C}$ translocated out of the treated second-leaf. Bentazon, however, did not influence the amount of $^{14}\text{C}$ from $^{14}\text{C}$-mefluidide that was translocated to the third leaf. The decrease in the basipetal translocation into root and first-leaf might have resulted from changes in physiological plant processes due to severe necrosis of the plant in combination treatments. This overall decrease in the translocation of $^{14}\text{C}$ from $^{14}\text{C}$-mefluidide in combination treatment, therefore, cannot account for the observed synergism. Our results do not agree with the observations of Eastin (10) who reported that bentazon did not influence the movement of mefluidide. The
differences between our results and the results of Eastin could be due
to differences in the placement of the herbicides. Eastin sprayed the
entire plant with the mixture of mefluidide and bentazon whereas we have
sprayed the plants with either mefluidide or bentazon.

There was significantly less translocation of radioactivity from
\(^{14}\text{C}-\text{bentazon}\) from the treated leaf when used alone than when used in
combination with mefluidide. The limited translocation of bentazon,
when used alone, could partly be explained because of its rapid metabo-
lism and conjugation in red rice as such processes occur in tolerant
cultivated rice (23). The tolerant cultivars of soybeans were also
reported to translocate less bentazon and/or its metabolites as compared
to sensitive cultivars (32). The addition of mefluidide might have
resulted in decreased metabolism and conjugation of bentazon and conse-
quently increased the translocation of radioactivity from \(^{14}\text{C}-\text{bentazon}\)
to first and third leaves. No visual symptoms of injury, however, were
seen either on first- or third-leaf in the combination treatment although
the amount of \(^{14}\text{C}\) present in these leaves was significantly higher than
when \(^{14}\text{C}-\text{bentazon}\) was applied alone. The \(^{14}\text{C}\) that is translocated to
the first- and third-leaf may be from its nontoxic metabolite(s), or the
concentrations of bentazon may have been too low to produce toxic symp-
toms. Our results agree with the reports of Eastin (10) who also found
increased translocation of \(^{14}\text{C}\) from \(^{14}\text{C}-\text{bentazon}\) throughout the entire
plant due to addition of mefluidide. Because of lack of necrosis other
than on treated-leaf, our observations indicate that this increased
translocation does not result in greater phytotoxicity as Eastin (10)
concluded. Mahoney and Penner (20) also reported that the extent of
translocation did not relate to the susceptibility of common cocklebur

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The differences in the loss, uptake, and translocation of \(^{14}\text{C}-\text{mefluidide}\) due to addition of bentazon and of \(^{14}\text{C}-\text{bentazon}\) due to addition of mefluidide were significant, however, they could not account for the observed synergism in the combination treatments. The uptake of both herbicides by red rice was less in the combination treatments than when applied alone. Since no injury symptoms were seen on the leaves above and below the treated leaf, the translocated radioactivity of \(^{14}\text{C}-\text{bentazon}\) in the combination treatment is believed to be its nonphytotoxic metabolite(s) rather than the active bentazon molecule. Therefore, it is postulated that the synergism of mefluidide-bentazon mixture on red rice is not entirely related to the amount of either herbicide absorbed into the leaf, retained, or translocated within the plant but mostly to the differences in the metabolism of these herbicides by red rice.

ACKNOWLEDGMENTS

The authors acknowledge the partial financial support by the Louisiana Soybean Promotion Board, BASF Corp., and 3M Company. The authors also express their appreciation to L. Lyons and T. Terrell for their technical assistance, J. H. Thompson for statistical analysis, and K. L. Koonce, E. P. Dunigan, L. M. Kitchen, and J. B. Baker for review of this manuscript.
LITERATURE CITED


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Mechanism of Interaction Between Mefluidide and Bentazon on Red Rice (Oryza sativa). II.

Metabolism of the Herbicides

SUDABATHULA RAO and T.R. HARGER

Abstract. The metabolism and degradation of mefluidide \( N-[2,4\text{-dimethyl-5-}[[\text{trifluoromethyl} \text{ sulfonyl}] \text{amino}] \text{phenyl} \text{acetamide}] \) and bentazon \( [3\text{-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one \ 2, 2\text{-dioxide}}] \) when applied alone and in combinations as well as the toxicity of the herbicide metabolites on red rice (Oryza sativa L. 'Strawhulled') were investigated. Mefluidide applied alone reduced the height while bentazon alone did not affect red rice. The combination of these two herbicides caused severe necrosis and death of red rice. Radioactive herbicides were utilized in order to isolate and identify the herbicides and their metabolites. The herbicide combination resulted in significant reduction in their degradation as compared to when they were applied alone. There were no significant changes in the metabolism of mefluidide by red rice due to addition of bentazon. Bentazon, when

1Received for publication.

This is a portion of the senior author's dissertation for the Ph.D. degree.

applied alone, was partly hydrolyzed to 6-hydroxy-bentazon. Both bentazon and its metabolite were rapidly conjugated into water-soluble compounds. Methanol-HCl hydrolysis of the water-soluble compounds yielded bentazon and 6-hydroxy-bentazon. Mefluidide prevented the conjugation and maintained high levels of free bentazon inside the treated red rice. In sequential application studies where the treatment with mefluidide was delayed by 32 h following the application of bentazon, injury symptoms appeared one day after mefluidide application. Mefluidide prevented further conjugation of bentazon that continued to enter the plant following mefluidide application. The known plant metabolites of these two herbicides were not phytotoxic to red rice. Free bentazon and 6-hydroxy-bentazon were identified using known standards by thin-layer chromatography (TLC), gas chromatography (GC)-mass spectrometry (MS) and nuclear magnetic resonance (NMR). The basis for synergistic interaction between mefluidide and bentazon appears to be the inhibition of bentazon metabolism by red rice in the presence of mefluidide. Free and unmetabolized bentazon probably caused necrosis that led to the death of red rice.

Additional index words. Herbicide degradation, herbicide combination, sequential application, synergism, metabolite toxicity, herbicide conjugation.

INTRODUCTION

Synergistic control of red rice with foliar applications of mefluidide-bentazon combinations has been documented by several researchers (1, 9, 19). Some differences in the loss of mefluidide (20) and in the absorption (20) and translocation of both the herbicides by...
red rice have been reported due to combinations of mefluidide and bentazon (8, 20). But these differences could not account fully for the observed synergism (20). Rao and Harger (20) postulated the basis for synergism in red rice to altered metabolism of these herbicides when used in combination.

Metabolism studies with mefluidide reveal that tolerant soybeans [Glycine max (L.) Merr.] rapidly metabolize mefluidide to water-soluble products (3, 4, 10) whereas susceptible common cocklebur (Xanthium pensylvanicum Wallr.) (3, 4) and corn (Zea mays L.) (10) only slowly metabolize mefluidide. The mechanism of herbicidal action of mefluidide is not well understood. However, mefluidide was reported to inhibit RNA synthesis (6), protein synthesis (6, 23), and possibly interfere in IAA metabolism (6).

Bentazon was reported to be primarily a photosynthetic inhibitor (13, 18, 21) disrupting electron flow at Q and PQ complexes near photosystem II (22). The susceptible plants were reported to retain more herbicide spray (12, 17), absorb greater quantities of bentazon (11, 15), translocate bentazon throughout the plant (11, 12, 25, 26), and metabolize bentazon at a slower rate (11, 12, 13, 14, 17, 21) as compared to tolerant plants. Mine et al. (14) reported that tolerant rice (Oryza sativa L.) converted 85% of the absorbed bentazon to a major water-soluble metabolite, 6-(3-isopropyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide)-0-β-D-glucopyranoside, while the susceptible Cyperus serotinus Rottb. metabolized only 25 to 50% in 7 days. This slower rate of metabolism could be the basis for bentazon selectivity. The metabolic changes in mefluidide and bentazon due to combinations have not been reported to date.

The purpose of these investigations was to evaluate differences in
metabolism of mefluidide and bentazon when applied alone and in combinations on red rice as a possible mechanism for synergistic interactions.

MATERIALS AND METHODS

All red rice plants were germinated and grown in a greenhouse. Seed source, planting techniques, growing conditions, and cultural practices were the same as described by Rao and Harger (20).

Herbicide Degradation Studies. The degradation of $^{14}$C-bentazon + mefluidide and $^{14}$C-mefluidide + bentazon on the leaf surface of dead red rice plants was monitored. The purpose of this study was to determine the extent of physical and chemical decomposition of these herbicides as influenced by the environmental conditions in the greenhouse in order to enable to recognize these products from the metabolites on the surface of living red rice plant. Radioactive plus non-radioactive herbicides were applied to adaxial leaf surface of dead red rice plants, and wash and extract of shoot were prepared at various days after treatment (DAT) as previously described under uptake study (20). The plant washes and extracts were pooled, acetone and methanol were evaporated on a rotary evaporator, and a 1-ml aliquot was assayed for radioactivity. The remaining water phase was shell-frozen in a dry ice and acetone bath and freeze-dried. The residue was redissolved in 5 ml methanol, condensed to 0.5 ml under $\text{N}_2$, and the entire sample was subjected to separation by TLC. After TLC separation, the radioactive zones (above 500 dpm) on TLC plates were visualized using a radiochromatogram spark chamber (Birchover Instruments, Ltd). The silica gel was removed from the plates for each individual zone and assayed for radioactivity.

Metabolism of $^{14}$C-mefluidide and $^{14}$C-bentazon. The metabolism of
$^{14}$C-mefluidide when used alone or in combination with bentazon as well as the metabolism of $^{14}$C-bentazon when used alone or in combination with mefluidide by living red rice plants was studied and compared. Wash and extract of shoots of herbicide-treated red rice were prepared at various DAT as previously described under uptake study (20). The plant washes and extracts were separately condensed to 0.5 ml as described in the degradation study, and the entire samples were subjected to separation by TLC. The radioactive zones were visualized, and the silica gel in these zones was removed and assayed for radioactivity as described earlier.

**Sequential mefluidide application study.** The metabolism studies indicate that when bentazon is used alone, all the radioactivity from $^{14}$C-bentazon which enters the red rice plant is metabolized to polar compounds within two days. However, when the application of mefluidide is delayed by four days following the application of bentazon, there still is synergistic growth reduction of red rice (19), although there is no free bentazon inside the plant. The purpose of this study was to determine if bentazon metabolism is a reversible process and if mefluidide could reverse this process to release free toxic bentazon in red rice.

Red rice plants at the 3 leaf-stage (18 to 22 cm) were sprayed with commercial formulation of bentazon followed by foliar application of $^{14}$C-bentazon at a total herbicide rate of 0.6 kg/ha as previously described under herbicide loss study (20). The experiment consisted of three treatments. In treatments 1 and 2, the plants were harvested at 32 and 96 hours after treatment (HAT), respectively. In treatment 3, red rice plants were sprayed with the commercial formulation of mefluidide at 0.3 kg/ha at 32 h following the application of $^{14}$C-bentazon + bentazon. The plants in treatment 3 were also harvested along with the
plants in treatment 2. Visual observations were made before each harvest. The plants were harvested and handled the same way as previously described in uptake study (20) and as summarized in Figure 1. The foliar wash and shoot extract were subjected through several steps of purification to isolate and quantify the plant metabolites.

The methanol shoot extract was condensed to 5 ml on a rotary evaporator and then to 0.5 ml under N$_2$ from which a sample was removed for TLC (Figure 1,C). The remainder was evaporated to dryness under N$_2$. The residue was redissolved in 50 ml water (Figure 1,D), the solution was acidified to pH 3.0, and partitioned three times each with 50 ml of ethyl acetate using 125-ml separatory funnels (Figure 1,E). The pH of the ethyl acetate phase was adjusted to 7.0, a 1-ml aliquot was assayed for the radioactivity, and the remainder was condensed to 0.5 ml using a rotary evaporator and N$_2$ (Figure 1,F). The entire sample was subjected to separation by TLC (Figure 1,G). The pH of the water phase from ethyl acetate partitioning of shoot extract was adjusted to 7.0, a 1-ml aliquot was removed for radioactivity assay, and the remainder was freeze-dried. The residue was redissolved in 5 ml methanol, condensed to 0.5 ml under N$_2$ from which a sample was subjected to TLC (Figure 1,P), and the remainder was dried under N$_2$. The residue was refluxed with 20 ml 4N methanolic hydrochloric acid for 90 min at 55 C under anhydrous conditions to hydrolyze the polar conjugates (Figure 1,Q). The pH of the hydrolyzed material was readjusted to 7.0, condensed to 0.5 ml using a rotary evaporator and N$_2$ (Figure 1,R), and the entire sample was subjected to separation by TLC (Figure 1,G). After TLC separation, the radioactive zones on TLC plates were visualized, and the silica gel was removed (Figure 1,H) and assayed for radioactivity.
Figure 1. Isolation, quantification, and identification procedure for $^{14}$C-mefluidide, $^{14}$C-bentazon, and their metabolites in red rice.
The foliar wash was also carried through several steps of analysis as shown in Figure 1. After redissolving the residue in 5 ml methanol and condensed to 0.5 ml under N\textsubscript{2}, a sample was subjected to TLC (Figure 1,S), and the remainder was dried under N\textsubscript{2}. The residue was redissolved in 50 ml water (Figure 1,T) and subjected through same steps of analysis (Figure 1,D) as described before for methanol shoot extract.

**Isolation and identifcation of bentazon and its metabolites.**

This study was conducted in order to isolate large quantities of bentazon and its metabolites from $^{14}$C-bentazon + bentazon + mefluidide treatments in order to enable identification utilizing MS and NMR. A total of 200 plants were utilized per each treatment at the rate of 20 plants per pot. Red rice plants at 3 leaf-stage were treated with commercial formulations of bentazon + mefluidide on a greenhouse belt sprayer as previously described under herbicide loss study (20). Immediately following, a total of 10 $\mu$Ci of $^{14}$C-bentazon per treatment was placed randomly on the adaxial leaf surface of all the plants. The total rates of herbicides applied were 0.3 kg/ha for mefluidide and 0.6 kg/ha for bentazon. Plants were harvested at the soil level 4 DAT and only the shoot was used for analysis because of limited translocation of bentazon into the root (20). The shoots were washed (Figure 1,A) to remove unabsorbed chemicals, and the washed shoots were homogenized, extracted with methanol, and filtered (Figure 1,B) as described previously. The shoot extract was subjected through several steps of purification as described in sequential mefluidide application study and shown in Figure 1. After the radioactive zones were scraped from TLC plates (Figure 1,H), the silica gel was eluted with 15 ml each of ethyl acetate, methanol, and acetone sequentially (Figure 1,I) and filtered through millipore filters (Millipore,
th (Figure 1,J). The filtrate was evaporated to dryness under N₂. The residues were incubated with 20 ml of freshly-prepared alcohol-free ethereal solutions of diazomethane (Diazald, Aldrich) (Figure 1,K) for 24 h in an ultrasonicator and the excess diazomethane was evaporated under N₂. The residues were redissolved in 1-ml of acetone (Figure 1,L) or CO(CD₃)₂ (Figure 1,M) and the samples were subjected to GC-MS (Figure 1,N) and NMR (Figure 1,O) analysis.

**Thin-layer chromatography.** Disposable micropipettes were used to spot 100 to 500 μl of extract or wash at various stages of analysis to pre-coated silica gel glass plates (60 F-254, 0.25 mm, EM Laboratories, Inc.). All plant metabolites were cochromatographed with known standards of ¹⁴C-bentazon and metabolites of bentazon³ or ¹⁴C-mefluidide or metabolites of mefluidide⁴. Plates were developed 15 cm in ethyl acetate:glacial acetic acid (49:1, v/v) for ¹⁴C-mefluidide samples and dioxane:chloroform:ethyl alcohol (6:6:1, v/v/v) for ¹⁴C-bentazon samples.

**Radioactivity Assay.** The radioactivity in the samples was quantitated by liquid scintillation spectrometry (Beckman LS-250). All samples were corrected for quenching using a standard curve from channel ratio method and backround radiation. Solid residues were combusted in oxygen with an oxidizer (Harvey BMO) and ¹⁴CO₂ produced was trapped in 15 ml of prepared scintillation cocktail, Scintisorb-C (Isolab). Aqueous and nonaqueous liquid samples and silica gel from TLC plates were counted in 15 ml of prepared scintillation cocktail, Scintisol

³BASF Corp.
⁴3M Company.
GC-MS and NMR analysis. The methylated bentazon and 6-hyroxy-bentazon standards and unknowns were dissolved in acetone and CO(CD₃)₂ for GC-MS and NMR analysis, respectively. The methodology used for GC-MS analysis was a modification of that used by Otto et al. (16). One µl samples were injected into gas chromatograph with a flame ionization or a quadrupol MS as a detector. Details of instrumentation are as follows—GC: HP 5840A; detector: flame ionization; temperature: 250 C; column: glass, 25 m long, 0.2 mm i.d., packing: deactivated SP 2100 fused silica; temperature: 120 C increased at the rate of 4 C/min to 240 C; carrier gas: He, 1 ml/min; flame gasses: H₂, 30 ml/min, air, 250 ml/min; and injector temperature: 200 C. MS: HP 5985A with 2648A data system. Spectra were measured at 70 ev in the EI mode at 35-600 amu with electron multiplier quadrupol. NMR: Bruker WP 200; Lock: D; reference TMS; temperature: ambiant; SW: 4000 Hz/8K, 01: 4200 Hz, 50 Hz/cm; PW: 2 µsec; AQ: 1.00, and NS: 250 scans.

Metabolite toxicity study. The toxicity of mefluidide, bentazon, and the known metabolites of these herbicides when used alone or in combinations on red rice was studied. The treatments are given in Table 7. Red rice plants at the 3 leaf-stage (15 to 18 cm) were treated with 0.3 kg/ha of mefluidide or its known metabolites and/or 0.6 kg/ha of bentazon or its known metabolites along with a surfactant, alkyl aryl polyoxyethylene glycol at 0.25% (v,v) on a greenhouse belt sprayer as described in previous studies. Twenty plants per pot were utilized for

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5 Hewlett-Packard.
6 Triton Ag-98.
this study. All plants were harvested 21 DAT at the soil level after taking visual observations and height measurements. Fresh weight data of only the green shoots were recorded.

In all experiments, treatments were replicated four times in a completely randomized design and all experiments were repeated at least once. Isolation and identification of bentazon and its metabolites—study, however, was not repeated. The data were analyzed by analysis of variance and protected Fisher’s least significant difference was used for mean separation.

RESULTS AND DISCUSSION

Herbicide degradation studies. The results of TLC separation of degradation products of $^{14}$C-mefluidide and $^{14}$C-bentazon on dead red rice are presented in Tables 1 and 2, respectively. The addition of bentazon to $^{14}$C-mefluidide or mefluidide to $^{14}$C-bentazon did not cause any changes in the number of degradation products of the $^{14}$C-herbicides. The nature of the degradation products of these herbicides, however, could not be characterized. In general, there was an increase in the amount of unextractable radioactivity with an increase in time after treatment in the shoots of dead plants. There was no influence of one herbicide on the other on the amount of $^{14}$C in the residues. This increase of $^{14}$C in the residue could be due to some kind of chemical binding with the plant tissues caused by chemical-, photo-, or microbial-decomposition of herbicides. There was approximately 60% loss in the amount of radioactivity which cochromatographed with $^{14}$C-mefluidide (Rf 0.75) at 2 DAT with a continuous decline up to 6 DAT. The loss in radioactivity which cochromatographed with $^{14}$C-bentazon (Rf 0.73) was
Table 1. TLC separation of radioactivity from shoots of dead red rice plants at 0, 2, 4, and 6 DAT with $^{14}\text{C}$-mefluidide ± bentazon.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distribution of radioactivity$^a$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>At 0 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide</td>
<td>0.3</td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide + bentazon</td>
<td>0.6</td>
</tr>
<tr>
<td>At 2 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide</td>
<td>10.2</td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide + bentazon</td>
<td>9.8</td>
</tr>
<tr>
<td>At 4 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide</td>
<td>15.7</td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide + bentazon</td>
<td>16.2</td>
</tr>
<tr>
<td>At 6 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide</td>
<td>16.1</td>
</tr>
<tr>
<td>$^{14}\text{C}$-mefluidide + bentazon</td>
<td>13.2</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>2.3</td>
</tr>
</tbody>
</table>

$^a$Data were expressed as percentage of the total radioactivity placed on the plant.

$^b$Cochromatographed with 3-acetamido-4,6-dimethyl aniline standard.

$^c$Cochromatographed with mefluidide standard.
Table 2. TLC separation of radioactivity from shoots of dead red rice plants at 0, 2, 4, and 6 DAT with $^{14}$C-bentazon + mefluidide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distribution of radioactivity&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residue</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>At 0 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>0.6</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>0.4</td>
</tr>
<tr>
<td>At 2 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>5.8</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>6.0</td>
</tr>
<tr>
<td>At 4 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>22.5</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>20.8</td>
</tr>
<tr>
<td>At 6 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>26.4</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>26.2</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>2.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data were expressed as percentage of the total radioactivity placed on the plant.

<sup>b</sup>Cochromatographed with 8-hydroxy-bentazon standard.

<sup>c</sup>,<sup>d</sup>Cochromatographed with bentazon and 6-OH-bentazon, respectively.
approximately 33% in 2 days and the losses continued until the experiment was terminated at 6 DAT. This decrease may have been due to photodegradation, volatilization, or microbial degradation of these herbicides. The combination of these herbicides resulted in significant decrease in the degradation of each as measured at 2, 4, and 6 DAT. The decrease in the degradation of both herbicides due to combinations may involve some physical binding with each other and with the used surfactant which may make this complex more stable to decomposition than when the herbicides were used alone. The amount of $^{14}$C that remained at the origin of TLC plates increased up to 2 DAT and thereafter remained essentially constant. This material at the origin may be a mixture of several degradation products that are polar in nature. The addition of bentazon decreased the amount of radioactivity from mefluidide at the origin; yet mefluidide failed to reduce the amount of $^{14}$C from bentazon at the origin. This indicates that mefluidide does not have an influence on the process that converts bentazon to a polar material which remains at the origin of TLC plates.

The degradation product of mefluidide with Rf value of 0.58 was cochromatographed with a known plant metabolite, 3-acetamido-4,6-dimethyl aniline. The products of degradation of bentazon with Rf values of 0.58 and 0.91 were cochromatographed with known plant metabolites, 8-hydroxy-bentazon and 6-hydroxy-bentazon, respectively. Since plant metabolites could not be expected on dead plants, those could possibly be the metabolites of microbial degradation. There were no differences in the amounts of these metabolites when the herbicides were used alone or in combination with each other. There were unidentified degradation products from both mefluidide (Rf 0.17 and 0.36) and bentazon (Rf 0.14),
the amounts of which, however, did not differ when these herbicides were used alone or in combination with each other. There is no literature available on the number and nature of degradation products of mefluidide except that mefluidide is reported to be susceptible to photodecomposition in solution (24). But bentazon was reported to be decomposed readily when exposed to high temperature, ultraviolet radiation (24), or when stored in aqueous solutions for prolonged periods (5). Our results agree with those of Eastin (7) who reported that photolysis of bentazon gave at least four breakdown products separated by TLC.

These studies indicate that there was no influence of one herbicide on the other on the total number and the nature of degradation products. It should, however, be noted that only the Rf values were utilized to determine the total number and nature of the degradation products of these herbicides in these studies.

Metabolism of $^{14}$C-mefluidide and $^{14}$C-bentazon. The results of the analysis of TLC separation of radioactivity in foliage wash of red rice plants treated with $^{14}$C-mefluidide and $^{14}$C-bentazon are presented in Tables 3 and 4 respectively. The $^{14}$C-compounds present on the surface of treated red rice had the same Rf values as the degradation products found on dead plants in the previous study (Tables 1 and 2). Since the total number and Rf values of these products are the same between this and previous study, these products may have been formed by some means other than plant metabolism such as photodecomposition and microbial degradation. Further, the symptoms of severe leaf necrosis were first noted one day after the treatment when the herbicides were used in combination (20.). Therefore, in the combination treatments, normal plant metabolism was not occurring. Since degradation also occurred in
Table 3. TLC separation of radioactivity in foliage wash of red rice plants at 0, 2, 4, and 6 DAT with $^{14}$C-mefluidide + bentazon.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Origin</th>
<th>Rf</th>
<th>Rf</th>
<th>Rf</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.17</td>
<td>0.36</td>
<td>0.58$^b$</td>
<td>0.75$^c$</td>
</tr>
<tr>
<td><strong>At 0 DAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>3.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>96.3</td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>3.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>96.0</td>
</tr>
<tr>
<td><strong>At 2 DAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>8.1</td>
<td>0.0</td>
<td>2.1</td>
<td>4.0</td>
<td>11.9</td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>12.7</td>
<td>0.0</td>
<td>2.4</td>
<td>6.1</td>
<td>14.7</td>
</tr>
<tr>
<td><strong>At 4 DAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>7.8</td>
<td>0.0</td>
<td>2.8</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>11.8</td>
<td>0.0</td>
<td>2.9</td>
<td>5.8</td>
<td>13.5</td>
</tr>
<tr>
<td><strong>At 6 DAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>5.3</td>
<td>3.0</td>
<td>1.9</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>7.0</td>
<td>3.2</td>
<td>3.0</td>
<td>4.6</td>
<td>10.8</td>
</tr>
<tr>
<td><strong>LSD 0.05</strong></td>
<td></td>
<td>0.9</td>
<td>0.3</td>
<td>1.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Data were expressed as percentage of the total radioactivity placed on the plant.

$^b$ Cochromatographed with 3-acetamido-4,6-dimethyl aniline standard.

$^c$ Cochromatographed with mefluide standard.
Table 4. TLC separation of radioactivity in foliage wash of red rice plants at 0, 2, 4, and 6 DAT with $^{14}\text{C}$-bentazon + mefluidide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distribution of radioactivity$^a$</th>
<th>Origin</th>
<th>Rf</th>
<th>Rf</th>
<th>Rf</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
<td>0.58$^b$</td>
<td>0.73$^c$</td>
<td>0.91$^d$</td>
</tr>
<tr>
<td>At 0 DAT</td>
<td></td>
<td></td>
<td>5.4</td>
<td>0.0</td>
<td>0.0</td>
<td>93.1</td>
</tr>
<tr>
<td>$^{14}\text{C}$-bentazon</td>
<td></td>
<td></td>
<td>5.4</td>
<td>0.0</td>
<td>0.6</td>
<td>8.9</td>
</tr>
<tr>
<td>+ mefluidide</td>
<td></td>
<td></td>
<td>4.1</td>
<td>0.0</td>
<td>0.0</td>
<td>93.0</td>
</tr>
<tr>
<td>At 2 DAT</td>
<td></td>
<td></td>
<td>7.3</td>
<td>0.0</td>
<td>0.5</td>
<td>19.0</td>
</tr>
<tr>
<td>$^{14}\text{C}$-bentazon</td>
<td></td>
<td></td>
<td>10.9</td>
<td>2.7</td>
<td>0.6</td>
<td>10.7</td>
</tr>
<tr>
<td>+ mefluidide</td>
<td></td>
<td></td>
<td>10.9</td>
<td>2.7</td>
<td>0.6</td>
<td>10.7</td>
</tr>
<tr>
<td>At 4 DAT</td>
<td></td>
<td></td>
<td>5.3</td>
<td>0.8</td>
<td>0.7</td>
<td>3.5</td>
</tr>
<tr>
<td>$^{14}\text{C}$-bentazon</td>
<td></td>
<td></td>
<td>9.4</td>
<td>3.0</td>
<td>1.0</td>
<td>8.0</td>
</tr>
<tr>
<td>+ mefluidide</td>
<td></td>
<td></td>
<td>9.4</td>
<td>3.0</td>
<td>1.0</td>
<td>8.0</td>
</tr>
<tr>
<td>At 6 DAT</td>
<td></td>
<td></td>
<td>5.1</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>$^{14}\text{C}$-bentazon</td>
<td></td>
<td></td>
<td>9.4</td>
<td>3.0</td>
<td>1.0</td>
<td>8.0</td>
</tr>
<tr>
<td>+ mefluidide</td>
<td></td>
<td></td>
<td>9.4</td>
<td>3.0</td>
<td>1.0</td>
<td>8.0</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td></td>
<td></td>
<td>1.2</td>
<td>0.3</td>
<td>0.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$ Data were expressed as percentage of the total radioactivity placed on the plant.

$^b$ Cochromatographed with 8-OH-bentazon standard.

$^c,d$ Cochromatographed with bentazon and 6-OH-bentazon, respectively.

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or on dead plants in the previous study, it is not possible to distinguish degradation occurring because of plant metabolism from that caused by microbial or chemical means. The amounts of products of degradation were, however, much lower on the surface of living plants in this study as compared to dead plants in the previous study. The degradation of herbicides that occur from the onset of necrotic symptoms in the combination treatments would be similar to that found on dead plants. Microorganisms could play a significant role in degrading herbicides from a dying tissue.

There was significant decrease in the total amount of $^{14}$C-herbicides on the surface of treated red rice with time. This decrease, however, may be contributed due to the sum of volatilization loss, entry into the plant, and degradation to simpler compounds. The fact that the amounts of $^{14}$C-herbicides remaining on the plant surface were much higher when these herbicides were used in combination than when they were applied alone may be due to decreased entry into the plant, loss from plant surface (20), and degradation into other compounds in the combination treatments. The amounts of $^{14}$C-herbicides present on the surface of living plants in this study were much lower as compared to dead plants in the previous study as a result of entry of the herbicides into the living plants, and other factors. Since the degradation products present on the plant surface appear to be the result of factors other than plant metabolism, the observed differences in the amounts of these products between single and combination treatments cannot be attributed to synergistic interactions of these herbicides inside the plant. These observed differences in the amounts of degradation products of these two herbicides between single and combination treatments might have arisen as a

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result of the washing techniques employed. Only the plants in the combi-
nation treatments had necrotic flecks, and water wash followed by acetone
rinse of the foliage might not have removed all of the radioactivity
present on the plant surface. Or this washing procedure could have
resulted in removal of the $^{14}\text{C}$ leaked out from inside the plant through
the necrotic flecks.

The same metabolites of $^{14}\text{C}$-mefluidide were noted in the shoot
extracts of red rice at various sampling dates when $^{14}\text{C}$-mefluidide was
used alone or in combination with bentazon (Table 5). There were no
differences in the amount of free mefluidide or its metabolites when
mefluidide was used alone or in combination with bentazon at two-day
sampling. The amount of $^{14}\text{C}$ in the polar materials at the origin of TLC
plates was significantly less when $^{14}\text{C}$-mefluidide was used in combination
with bentazon than when it was used alone. This decrease could be due to
less conjugation of mefluidide or its metabolites with sugar moiety as
reported earlier with mefluidide-susceptible species (2). There were no
differences in the amount of unextractable radioactivity between $^{14}\text{C}$-
mefluidide and $^{14}\text{C}$-mefluidide + bentazon (20). Although the differences
existed in the amounts of free mefluidide or its metabolites at 4 and 6
DAT between $^{14}\text{C}$-mefluidide and $^{14}\text{C}$-mefluidide plus bentazon treatments,
these differences might not have contributed to synergism of these
herbicides as visual symptoms of injury were noted even before the
measurable differences in the amounts of $^{14}\text{C}$-compounds were recorded.
The major metabolite (Rf 0.58) of mefluidide in red rice was identified
as 3-acetamido-4,6-dimethyl aniline (Metabolite 2) by comparing the Rf
values with the known standards. It is possible, however, that this
metabolite may actually be a photolysis or microbial degradation product.
Table 5. TLC separation of radioactivity in methanol extract of shoots of red rice plants at 0, 2, 4, and 6 DAT with $^{14}$C-mefluidide + bentazon.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distribution of radioactivity$^a$ (%)</th>
<th>Origin</th>
<th>Rf 0.17</th>
<th>Rf 0.58$^b$</th>
<th>Rf 0.75$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 0 DAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>0.4</td>
<td>0.0</td>
<td>0.0</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>At 2 DAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>14.2</td>
<td>0.0</td>
<td>6.5</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>11.5</td>
<td>0.0</td>
<td>6.4</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>At 4 DAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>17.7</td>
<td>3.0</td>
<td>7.7</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>11.2</td>
<td>0.8</td>
<td>7.1</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>At 6 DAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide</td>
<td>19.0</td>
<td>2.7</td>
<td>4.3</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-mefluidide + bentazon</td>
<td>9.9</td>
<td>1.9</td>
<td>3.7</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>1.0</td>
<td>0.3</td>
<td>0.7</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Data were expressed as percentage of the total radioactivity placed on the plant.

$^b$Cochromatographed with 3-acetamido-4,6-dimethyl aniline standard.

$^c$Cochromatographed with mefluidide standard.
of mefluidide that entered the plant. The presence of this metabolite in tolerant soybeans was reported earlier by Bloomberg and Wax (4).

Mefluidide had a profound influence on the metabolism of bentazon by red rice (Table 6). There was more radioactivity from $^{14}$C-bentazon in the unextractable residue when it was used in combination with mefluidide than when used alone (20). When bentazon was applied alone, almost all of the $^{14}$C was recovered in the polar materials at the origin and Rf 0.11. But when mefluidide was added, there was significantly less $^{14}$C from bentazon in these polar materials and large amounts of $^{14}$C were recovered as free bentazon (Rf 0.73), 6-hydroxy-bentazon (Rf 0.91), and 8-hydroxy-bentazon (Rf 0.58). There were no differences in the amounts of a metabolite at Rf 0.25 between $^{14}$C-bentazon and $^{14}$C-bentazon plus mefluidide. Since the metabolite at Rf 0.25 and 8-hydroxy-bentazon are not reported earlier with bentazon-treated cultivated white rice (Oryza sativa L.) (16), they may be the photolysis or microbial degradation products that entered the plant, the natural plant constituents that incorporated the $^{14}$C, or a new metabolite (Rf 0.58) that formed due to interaction with mefluidide. The polar materials were reported to contain natural plant constituents such as lignin, cellulose, starch, and protein that have incorporated $^{14}$C (16) as well as a conjugate, 6-(3-isopropyl-2,1,3-benzothiadiazin-4-one 2,2-dioxide)-O-β-glucopyranoside (14), which could be cleaved to yield 6-hydroxy-bentazon and glucose with β-oxidase or methanolic-HCl hydrolysis (16). The possible conjugation of bentazon with an unknown moiety in the presence of water was also reported by Booth et al. (5). This bentazon conjugate might have also contributed to the total $^{14}$C present at the origin and Rf 0.11 on TLC.

These results suggest that mefluidide is capable of either preventing the metabolism of $^{14}$C-bentazon to two nontoxic polar conjugates
Table 6. TLC separation of radioactivity in methanol extract of shoots of red rice plants at 0, 2, 4, and 6 DAT with $^{14}$C-bentazon + mefluidide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distribution of radioactivity $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Origin</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>At 0 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>0.9</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>0.3</td>
</tr>
<tr>
<td>At 2 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>31.9</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + bentazon</td>
<td>11.7</td>
</tr>
<tr>
<td>At 4 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>31.4</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>8.1</td>
</tr>
<tr>
<td>At 6 DAT</td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-bentazon</td>
<td>31.6</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td>9.0</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>1.9</td>
</tr>
</tbody>
</table>

$^a$Data were expressed as percentage of the total radioactivity placed on the plant.

$^b$Cochromatographed with 8-OH-bentazon standard.

$^c,d$Cochromatographed with bentazon and 6-OH-bentazon, respectively.
(origin and Rf 0.11) or reversing the metabolism of bentazon by breaking one or both of the polar conjugates to form toxic materials, such as bentazon or metabolites at Rf 0.58 and 0.91. The metabolites, 6-hydroxy-bentazon (Rf 0.91) and 8-hydroxy-bentazon (Rf 0.58), however, were reported to be less toxic than bentazon to treated susceptible plants (21).

**Sequential mefluidide application study.** In earlier studies (19), red rice plants showed visual symptoms of injury even when the application of mefluidide was delayed by 2 to 8 days following the application of bentazon. But the metabolism studies show that red rice metabolizes nearly all of the bentazon as soon as the herbicide enters the plant. It could not be determined from the previous studies whether mefluidide breaks polar conjugates of bentazon to release toxic bentazon or prevents the metabolism of bentazon that continues to enter the plant following the delayed application of mefluidide. This study was designed to determine if mefluidide breaks the polar conjugates of bentazon to release any bentazon or its toxic metabolites.

Red rice did not show visual symptoms of injury either at 32 or 96 HAT with $^{14}$C-bentazon alone. But when mefluidide was sprayed 32 HAT with $^{14}$C-bentazon, necrotic flecks started to develop one day after mefluidide spray and necrosis was severe covering approximately 60% of the leaf area at the time of harvest in the sequential treatment.

There was an increase in total uptake of $^{14}$C by red rice up to 96 HAT when $^{14}$C-bentazon was used alone (Table 7). But with the sequential application of mefluidide, there still was a significant uptake of $^{14}$C between 32 and 96 HAT, although the total uptake was less at 96 HAT as compared to when $^{14}$C-bentazon was used alone. The observed decrease in the uptake and total amount of $^{14}$C inside the plant between 32 and 96
Table 7. Recovery of radioactivity from red rice plants treated with $^{14}$C-bentazon + mefluidide in sequential mefluidide application study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distribution of radioactivity(^a)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wash</td>
<td>Extract</td>
</tr>
<tr>
<td>$^{14}$C-bentazon at 32 HAT</td>
<td>49.3</td>
<td>29.9</td>
</tr>
<tr>
<td>$^{14}$C-bentazon at 96 HAT</td>
<td>13.0</td>
<td>52.0</td>
</tr>
<tr>
<td>$^{14}$C-bentazon + mefluidide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 96 HAT</td>
<td>18.4</td>
<td>37.6</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>2.5</td>
<td>2.4</td>
</tr>
</tbody>
</table>

\(^a\)Data were expressed as percentage of the total radioactivity placed on the plant.

\(^b\)NS denotes nonsignificant.
HAT following mefluidide application may have been due to localized necrosis as indicated by total $^{14}$C recovered in foliage wash and shoot extract plus residue. The observed increase in the amount of $^{14}$C in unextractable residue at 96 HAT following mefluidide application compared to treatment with $^{14}$C-bentazon alone at the same sampling time was probably the result of incorporation of $^{14}$C in cell walls of partially dead tissues in the combination treatment. There were no significant differences in the amount of $^{14}$C in the root among the three treatments because of limited translocation of bentazon (20).

The partitioning of radioactivity in foliage wash and shoot extracts resulted in the recovery of the compounds that remained at the origin of TLC plates and Rf 0.11 and 0.14 in the water fraction whereas the compounds with Rf values of 0.25, 0.58, 0.73, and 0.91 remained in ethyl acetate portion. Since there were no differences in the amounts of $^{14}$C at each individual radioactive zone on TLC plates between before and after partitioning, only the results of the analysis of separation of radioactivity on TLC of wash and extract before partitioning are presented in Table 8.

Most of the $^{14}$C recovered in water wash at 32 HAT was present as free bentazon. There was a significant decrease in the amount of free $^{14}$C-bentazon and $^{14}$C at Rf 0.14 at 96 HAT as compared to sampling at 32 HAT which may have been due to entry of the $^{14}$C into the plant. Mefluidide prevented the decrease of $^{14}$C at Rf 0.14 between 32 and 96 HAT without any significant affects on the amount of $^{14}$C present in other degradation products.

The radioactivity in the methanol extracts contained the same metabolites found in previous study. When mefluidide was sprayed at 32 HAT
Table 8. TLC separation of radioactivity in water wash and methanol extract of red rice plants treated with \(^{14}\text{C}-\text{bentazon} + \text{mefluidide}\) in sequential mefluidide application study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wash</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Origin</td>
<td>Rf</td>
</tr>
<tr>
<td>(^{14}\text{C}-\text{bentazon at 32 HAT})</td>
<td>0.14</td>
<td>0.58</td>
</tr>
<tr>
<td>(^{14}\text{C}-\text{bentazon at 96 HAT})</td>
<td>7.0</td>
<td>1.1</td>
</tr>
<tr>
<td>(^{14}\text{C}-\text{bentazon + mefluidide at 96 HAT})</td>
<td>9.6</td>
<td>3.1</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>NS(^{e})</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^{a}\)Data were expressed as percentage of the total radioactivity placed on the plant.

\(^{b, c, d}\)Cochromatographed with 8-OH-bentazon, bentazon, and 6-OH-bentazon standards, respectively.

\(^{e}\)NS denotes nonsignificant.
with $^{14}$C-bentazon, there was a significant lowering of the amount of $^{14}$C at the origin of TLC plates and prevention of the formation of metabolites at Rf 0.11. This treatment did not affect the amounts of other metabolites of $^{14}$C-bentazon, but maintained significantly higher levels of free $^{14}$C-bentazon inside red rice when compared to bentazon applied alone. These results indicate that the addition of mefluidide probably does not cause breakage of the polar conjugates of bentazon or its metabolites but only lowers or prevents the formation of polar conjugates of bentazon. The free bentazon that was present inside the plant in the combination treatment was probably the result of continued entry into the plant and that portion that remained unmetabolized due to effect of mefluidide.

The methanol-HCl hydrolysis of the polar compounds from the origin of TLC plates in the foliage wash yielded bentazon, 6-hydroxy-bentazon, 8-hydroxy-bentazon, an unknown compound at Rf 0.14 along with unknown polar complex materials that could not be cleaved (Table 9). The $^{14}$C at Rf 0.14 could not be cleaved by hydrolysis and this material did not move from the origin of TLC plate. These polar compounds from foliage wash that could not be cleaved by hydrolysis may be the products of degradation that polymerized to form polar complexes.

The $^{14}$C obtained from the origin of TLC plates in shoot extract also yielded bentazon, 6-hydroxy-bentazon, and an unknown compound at Rf 0.11 upon hydrolysis (Table 9). A significant portion of the $^{14}$C, however, remained at the origin of the TLC plate. The $^{14}$C at Rf 0.11 also released free bentazon, 6-hydroxy-bentazon, and two unknown complexes that remained one each at the origin and at Rf 0.11. These polar compounds from the shoot extract appear to be different from those in foli-
Table 9. Methanol-HCl hydrolysis of polar materials from wash and extract of red rice plants treated with $^{14}$C-bentazon + mefluidide in sequential mefluidide application study.

<table>
<thead>
<tr>
<th>Materials hydrolyzed</th>
<th>Distribution of radioactivity after hydrolysis$^a$</th>
<th>Wash</th>
<th></th>
<th></th>
<th>Extract</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Origin Rf Rf Rf Origin Rf Rf Rf Origin Rf Rf Rf</td>
<td>0.11</td>
<td>0.58</td>
<td>0.73</td>
<td>0.91</td>
<td>0.11</td>
<td>0.73</td>
<td>0.91</td>
</tr>
<tr>
<td>Origin</td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-b at 32 HAT</td>
<td>1.6 2.2 1.1 1.5 1.7</td>
<td>7.8</td>
<td>3.8</td>
<td>7.2</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-b at 96 HAT</td>
<td>1.1 1.9 0.9 1.6 1.5</td>
<td>10.4</td>
<td>3.5</td>
<td>10.8</td>
<td>5.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-b + m at 96 HAT</td>
<td>1.3 3.0 1.3 2.0 2.0</td>
<td>20.2</td>
<td>1.5</td>
<td>3.7</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>NS$^e$ NS$^e$ NS$^e$ NS$^e$ NS$^e$</td>
<td>1.5</td>
<td>0.4</td>
<td>1.1</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rf 0.14 or 0.11$^f$</td>
<td>3.0 0.0 0.0 0.0 0.0</td>
<td>1.5</td>
<td>0.7</td>
<td>2.6</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-b at 32 HAT</td>
<td>1.1 0.0 0.0 0.0 0.0</td>
<td>5.9</td>
<td>2.2</td>
<td>8.0</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-b at 96 HAT</td>
<td>3.1 0.0 0.0 0.0 0.0</td>
<td>5.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.5 NS$^e$ NS$^e$ NS$^e$ NS$^e$</td>
<td>1.0</td>
<td>0.5</td>
<td>1.1</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Data were expressed as percentage of the total radioactivity placed on the plant.

$^b,c,d$Cochromatographed with 8-OH-bentazon, bentazon, and 6-OH-bentazon standards, respectively.

$^e$NS denotes nonsignificant.

$^f$Rf 0.14 and 0.11 refer to radioactivity from wash and extract, respectively.
age wash because of differences in the number and amounts of metabolites that are conjugated. The amount of free bentazon that was released upon hydrolysis of polar conjugates at the origin or Rf 0.11 was significantly lower than when bentazon was applied alone. This may be due to less conjugation in the combination treatment. The radioactivity that remained at or close to the origin of TLC plates in the shoot extract after hydrolysis was probably in glucose and other natural plant metabolites (16).

These results indicate that the polar $^{14}$C materials contain at least two conjugates, one each of bentazon and 6-hydroxy-bentazon, and probably natural plant metabolites that have incorporated $^{14}$C into them along with unknown complexes. The mefluidide combination appears to prevent the metabolism of bentazon into polar conjugates and maintains high levels of free bentazon. It should be noted, however, that there may be some contamination between foliage wash and shoot extracts due to incomplete or excessive washing of the foliage.

**Isolation and identification of bentazon and its metabolites.** The compounds that had Rf values of 0.73 on TLC plates from shoot extract, foliage wash, and hydrolysis of conjugates from shoot extract were identified as bentazon using known standards on GC-MS and NMR. The compound with Rf value of 0.73 had the same molecular weight (254) and similar breaking pattern on MS as bentazon standard after methylation. The compounds with Rf values of 0.91 had a molecular weight of 284 as 6-hydroxy-bentazon standard after methylation (16). The other compounds with Rf values of 0.11 and 0.58, however, could not be characterized. The compound at Rf 0.58, however, appears to be 8-hydroxy-bentazon based on TLC data.

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Metabolite toxicity study. Mefluidide and its metabolites 1 (4-amino-6-trifluoromethylsulfonyl-amino-m-xylene) and 3 (6-acetamide-4-nitro-m-xylene) applied alone resulted in significant growth reduction of red rice from untreated control (Table 10). Bentazon, 6-hydroxy-bentazon and 8-hydroxy-bentazon, however, were nontoxic to red rice. The combinations of 6-hydroxy-bentazon or 8-hydroxy-bentazon with mefluidide or metabolite 3 as well as bentazon with metabolites 1 or 3 have also resulted in significant height and fresh weight reduction of red rice from untreated control. Mefluidide and metabolite 1, when used alone or in combination with other compounds, caused the formation of callus growth near the base of the plant. The metabolites 1 and 3 of mefluidide, however, were not found in red rice in our studies. The most toxic treatment in this study was the combination of mefluidide and bentazon.

This study shows that the metabolites of either herbicide tested were only slightly toxic, and their activity does not account for the observed synergism of herbicide combination on red rice. So the phytotoxicant in the combination treatment appears to be free bentazon.

The mechanism of synergistic interactions between mefluidide and bentazon probably involves the prevention of bentazon metabolism by red rice through inhibitory effects of mefluidide on hydroxylases (21) which hydrolyze toxic bentazon to nontoxic 6-hydroxy-bentazon or through other unknown mechanisms.

ACKNOWLEDGMENTS

The authors acknowledge the partial financial support by the Louisiana Soybean Promotion Board. The authors extend their appreciation to BASF Corp., and 3M Company for the gift of radioactive chemicals,
Table 10. Toxicity of bentazon, mefluidide, and their metabolites when used alone and in combinations on red rice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm)</th>
<th>Fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>23</td>
<td>2.8</td>
</tr>
<tr>
<td>Bentazon</td>
<td>21</td>
<td>2.6</td>
</tr>
<tr>
<td>6-OH-bentazon</td>
<td>19</td>
<td>2.5</td>
</tr>
<tr>
<td>8-OH-bentazon</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>Mefluidide</td>
<td>16</td>
<td>2.0</td>
</tr>
<tr>
<td>Metabolite 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
<td>1.9</td>
</tr>
<tr>
<td>Metabolite 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>2.7</td>
</tr>
<tr>
<td>Metabolite 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>2.0</td>
</tr>
<tr>
<td>Bentazon + mefluidide</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bentazon + metabolite 1</td>
<td>14</td>
<td>1.7</td>
</tr>
<tr>
<td>Bentazon + metabolite 2</td>
<td>25</td>
<td>3.1</td>
</tr>
<tr>
<td>Bentazon + metabolite 3</td>
<td>16</td>
<td>1.9</td>
</tr>
<tr>
<td>Mefluidide + 6-OH-bentazon</td>
<td>13</td>
<td>1.4</td>
</tr>
<tr>
<td>Mefluidide + 8-OH-bentazon</td>
<td>14</td>
<td>1.6</td>
</tr>
<tr>
<td>Metabolite 1 + 6-OH-bentazon</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>Metabolite 1 + 8-OH-bentazon</td>
<td>22</td>
<td>2.6</td>
</tr>
<tr>
<td>Metabolite 2 + 6-OH-bentazon</td>
<td>21</td>
<td>2.9</td>
</tr>
<tr>
<td>Metabolite 2 + 8-OH-bentazon</td>
<td>22</td>
<td>2.8</td>
</tr>
<tr>
<td>Mefluidide 3 + 6-OH-bentazon</td>
<td>16</td>
<td>1.8</td>
</tr>
<tr>
<td>Mefluidide 3 + 8-OH-bentazon</td>
<td>15</td>
<td>1.8</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standards of known plant metabolites of mefluidide.
herbicides, and their metabolites. The authors also thank R. B. Shah
and N. H. Fischer for their technical assistance and K. L. Koonce,
J. B. Baker, E. P. Dunigan, and L. M. Kitchen for review of this
manuscript.
LITERATURE CITED


The responses of soybeans and red rice to postemergence applications of mefluidide and bentazon alone and in combinations were evaluated in greenhouse studies.

Soybeans were tolerant to bentazon up to the highest tested rate of 2.0 kg/ha, but their heights were reduced by mefluidide at 1.0 kg/ha. Soybeans were not injured by any tested combination of the two herbicides possibly due to antagonistic interactions.

Bentazon applied alone at rates of 5 kg/ha had no effect on red rice injury. Mefluidide applied at rates above 0.25 kg/ha severely reduced height and fresh weight of red rice. When these two herbicides were combined, a synergistic reaction occurred resulting in necrosis of treated plants, and the combination was much more effective in controlling red rice than was either herbicide applied alone. The addition of a surfactant significantly increased the activity of the combination on red rice up to 0.25 kg/ha of mefluidide and at all rates of bentazon.

The differences in degradation, uptake, translocation, metabolism, and unexplained loss of these two herbicides due to combinations with each other were studied in order to establish the basis for synergistic interactions of this herbicide combination on red rice.

The unexplained loss of $^{14}$C was assumed to be gaseous loss. This loss of radioactivity occurred from both living and dead red rice plants when the herbicides were used alone or in combination. The combinations of these herbicides resulted in less loss of mefluidide from living plants and of bentazon from dead plants than when they were applied alone. The decreased loss of mefluidide or bentazon when used in combination could be due to some kind of a physical binding of these
herbicides with each other and with the added surfactant or decreased metabolism of these herbicides by red rice to CO₂.

When the herbicide combination was used, there was less degradation and uptake of both mefluidide and bentazon than when either was used alone. The decreased loss of mefluidide, however, did not contribute to its increased uptake. The severe necrosis caused by the combination treatments probably restricted uptake of herbicides as compared to application of either herbicide where no symptoms of injury were seen. The translocation of both the herbicides from the treated second leaf was mainly acropetal with limited basipetal movement. The translocation of 14C-mefluidide was reduced when applied simultaneously with bentazon, however, the entire plant showed necrosis. The addition of mefluidide, on the other hand, increased the translocation of 14C from bentazon, but symptoms of injury were seen only on the treated leaf.

There were no significant differences in the metabolism of mefluidide by red rice when applied alone or in combination with bentazon. Bentazon applied alone was partly metabolized to 6-hydroxy-bentazon, and both bentazon and its metabolite were rapidly conjugated into two polar compounds. Simultaneous spraying with mefluidide lowered this conjugation and maintained high levels of free bentazon in red rice.

In studies with sequential applications of bentazon and mefluidide where mefluidide spray was delayed by 32 h following the application of bentazon, the injury symptoms were still evident. Although all the bentazon inside the plant, prior to the mefluidide application, was conjugated, mefluidide prevented further conjugation of bentazon that continued to enter the plant.

The known plant metabolites of these two herbicides were found to
be only slightly toxic and cannot account for observed necrosis on red rice. Although there were differences in loss, degradation, uptake, and translocation of the herbicides between single and combination treatments, they seem to contribute little to the observed synergism. Therefore, the basis for synergistic interactions between mefluidide and bentazon appears to be the inhibition of bentazon metabolism by red rice in the presence of mefluidide. The phytotoxic symptoms observed on treated red rice are consistent with the idea of photosynthetic inhibition in red rice by unmetabolized bentazon.


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102. Frear, D.S. and G.G. Still. 1968. The metabolism of 3,4-dichloro-
propionanilide in plants. Partial purification and properties of 
an aryl acylamidase from rice. Phytochemistry 7:913-920.

Ill. Abstr. 70.

29:108.


Sci. Soc. 29:60.

108. Glenn, D.S. 1979. Biochemical and physiological responses to me­

grass herbicides for soybean production. Proc. North Cent. Weed 
Control Conf. 32:30.


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183. McWhorter, C.G. and G.D. Wills. 1978. Factors affecting the translocation of 14C-mefluidide in soybeans (Glycine max), common cocklebur (Xanthium pensylvanicum), and johnsongrass (Sorghum halepense). Weed Sci. 26:382-388.


204. Nishimoto, R.K., A.P. Appleby, and W.R. Furtik. 1967. Site of up-
pp. 46-47.

205. Nishimura, M. and A. Takamiya. 1966. Energy and electron trans-
fer systems in algal photosynthesis. I. Action of two photo-
chemical systems in oxidation-reduction reactions of cytochrome

206. Oliver, L.R., W.M. Lambert, and W.D. Mathis. 1976. Overtop her-
ice applications for cocklebur control in soybean. Proc. South.

207. Olson, W.A. and J.D. Nalewaja. 1977. Factors affecting MCPA an-

Weed Sci. 30:42-43.

ence of herbicides for broad-leaved weeds and adjuvants with diclo-

bentazon (BAS 351-H) in soybeans: Experiments dealing with the
potential cleavage of bound metabolic complexes by rumen fluid.

211. Otto, S., P. Beutel, N. Drescher, and R. Huber. 1978. Investiga-
tions into the degradation of bentazon in plant and soil. Int.
Congr. Pestic Chem. 4:551-556.


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Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


259. 3M Company. 1978. Vistar 2-S-Postemergence Soybean Herbicide. EPA Experimental Use Permit. 3M Center, St. Paul, Minnesota.


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VITAE

The author was born on January 26, 1950 at Mandavalli, AP, India. He received his primary education in the same town. He graduated from High School from Andhra University, Waltair in March 1965. He, then, worked as a Farm Manager at Kottapalli, AP from April, 1965 to June, 1967 and during the same period, taught physical and biological sciences in High School. Later, he received a two year Associate degree in Agriculture from Uttar Pradesh Board, Allahabad, UP in June, 1969. He received a B.Sc. (Hons.) Ag. & A.H. with an elective in Crop Production in June, 1972 and a M.Sc. Ag. (Agronomy) in July, 1974 from G.B. Pant University of Agriculture & Technology, Pantnagar, UP. He was a recipient of Indian Council of Agricultural Research Merit Scholarship during his undergraduate studies and a graduate research assistantship during his graduate studies. He, then, joined Cornell University, Ithaca, NY as a graduate research assistant where he received a M.S. in Vegetable Crops in January, 1977. Later, he joined Louisiana State University, Baton Rouge, LA in February, 1977 as a Research Technician and accepted a graduate research assistantship in August, 1978 in the Department of Plant Pathology & Crop Physiology to work toward a Ph.D. degree.

The author is a member of Weed Science Society of America, Indian Society of Weed Science, Southern Weed Science Society, Louisiana Society for Electron Microscopy, Inc., and Gamma Sigma Delta.

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EXAMINATION AND THESIS REPORT

Candidate: Sudabathula Rajaramamohana Rao

Major Field: Plant Pathology

Title of Thesis: Interactions of Mefluidide and Bentazon on Red Rice (Oryza sativa L.)

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

October 29, 1981

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