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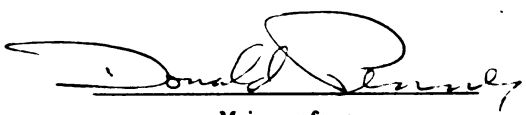
ABSORPTION, TRANSLOCATION, METABOLISM, AND MODE OF ACTION OF BUTHIDAZOLE(3-[5-(1,1-DIMETHYLETHYL)-1,3,4-THIADIAZOL-2-YL]-4-HYDROXY-1-METHYL-2-IMIDAZOLIDINONE) AS RELATED TO ITS SELECTIVITY BETWEEN CORN AND REDROOT PIGWEED AND BETWEEN ALFALFA AND QUACKGRASS

presented by

KRITON KLEANTHIS HATZIOS

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Weed Science



Major professor

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ABSORPTION, TRANSLOCATION, METABOLISM, AND MODE OF ACTION OF BUTHIDAZOLE (3-[5-(1,1-DIMETHYLETHYL)-1,3,4-THIADIAZOL-2-YL]-4-HYDROXY-1-METHYL-2-IMIDAZOLIDINONE) AS RELATED TO ITS SELECTIVITY BETWEEN CORN (*Zea mays* L.) AND REDROOT PIGWEED (*Amaranthus retroflexus* L.) AND BETWEEN ALFALFA (*Medicago sativa* L.) AND QUACKGRASS [*Agropyron repens* (L.) Beauv.]

By

Kriton Kleanthis Hatzios

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ABSTRACT

ABSORPTION, TRANSLOCATION, METABOLISM, AND MODE OF ACTION OF BUTHIDAZOLE (3-[5-(1,1-DIMETHYLETHYL)-1,3,4-THIADIAZOL-2-YL]-4-HYDROXY-1-METHYL-2-IMIDAZOLIDINONE) AS RELATED TO ITS SELECTIVITY BETWEEN CORN (Zea mays L.) AND REDROOT PIGWEED (Amaranthus retroflexus L.) AND BETWEEN ALFALFA (Medicago sativa L.) AND QUACKGRASS [Agropyron repens (L.) Beauv.]

By

Kriton Kleanthis Hatzios

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) is a promising new herbicide for selective weed control in corn (Zea mays L.) following preemergence or early postemergence applications and in established alfalfa (Medicago sativa L.) applied in fall or spring.

Absorption, translocation, metabolism, and mode of action were studied as potential factors contributing to buthidazole selectivity between tolerant corn and susceptible redroot pigweed (Amaranthus retroflexus L.) and between tolerant alfalfa and susceptible quackgrass [Agropyron repens (L.) Beauv.]

^{14}C-buthidazole was absorbed by both leaves and roots of all species and moved acropetally. Basipetal movement was evident only in redroot pigweed following foliar application. However, rapid uptake by the roots and rapid movement to the leaves via the xylem appeared to be the main pathway of uptake and translocation of ^{14}C-buthidazole in all species. Differential absorption and translocation did not appear to contribute to buthidazole selectivity between alfalfa and quackgrass. There were no differences in buthidazole absorption between corn and redroot pigweed. However, ^{14}C-buthidazole supplied to the roots was translocated more quickly to the redroot pigweed shoots and leaves. This may have contributed to the selectivity between corn and redroot pigweed.

Metabolism of ^{14}C-buthidazole was studied in the leaves and stems of all four plant species following root application and in corn following application to the emerging coleoptile. Alfalfa metabolized ^{14}C-buthidazole very rapidly yielding five metabolites beside the unmetabolized parent buthidazole. An unidentified metabolite with an Rf value of 0.21 (developing system Chloroform 80% : Methanol 20%) appeared to be the major metabolite in alfalfa accounting for 40% of the total radioactivity present in the leaves of treated plants 6 days after treatment. Metabolism of ^{14}C-buthidazole was very slow in quackgrass. Unmetabolized ^{14}C-buthidazole was present as 87% of the total radioactivity even after 6 days. Two metabolites with Rf values similar to those of the amine and dihydroxy derivatives of buthidazole were present in higher amounts in alfalfa as compared to quackgrass, whereas a metabolite with an Rf value similar to that of the methyl urea derivative of buthidazole was present only in alfalfa. Thus, differential rate of metabolism appeared to be a major factor contributing to buthidazole selectivity between alfalfa and quackgrass. Corn and redroot pigweed metabolized ^{14}C-buthidazole in a similar manner but at a different rate yielding as major metabolite an unidentified buthidazole derivative with Rf value of 0.24 to 0.26 in the chloroform:methanol (4:1) developing system. This metabolite appeared to be similar to the one detected in alfalfa. Corn formed this metabolite very rapidly even in roots, whereas the formation of this buthidazole derivative in redroot pigweed leaves and stems was slow. Minor metabolites detected in both corn and redroot pigweed appeared to be similar to the urea and dihydroxy derivatives by Thin Layer Chromatography (TLC). A metabolite with Rf value similar to the amine derivative of buthidazole was present only in corn leaves following application of labeled buthidazole to the emerging coleoptile. Thus, a differential rate of metabolism, combined with the faster movement of unmetabolized buthidazole from the roots to the

shoots and leaves of redroot pigweed appeared important in the selectivity of buthidazole between corn and redroot pigweed.

Time-course and concentration studies on the localization of the metabolic site of action of buthidazole were conducted with enzymatically isolated leaf cells from navy beans (Phaseolus vulgaris L.) and appropriate radioactive substrates. These studies revealed that photosynthesis was the most sensitive and first metabolic process inhibited by buthidazole at concentrations as low as 0.1 μM and as early as 30 min of incubation. Buthidazole at concentrations 10 and 100-μM also inhibited RNA and lipid syntheses, whereas protein synthesis was not affected by buthidazole at any concentration examined. Furhter studies with isolated spinach (Spinacea oleracea L.) chloroplasts indicated that inhibition of photosynthetic electron transport by buthidazole was primarily at the reducing side of photosystem II (between the unknown quencher Q and plastoquinone). A less inhibitory effect of buthidazole on the mechanism of water oxidation (oxidizing side of photosystem II) was also evident from the data. In vivo measurements of total photosynthesis and dark respiration of corn, redroot pigweed, alfalfa, and quackgrass plants treated with various rates of buthidazole applied postemergence were conducted by means of an infrared CO_2 analyzer at different time periods after application. The results confirmed the observations of the in vitro studies, suggesting that buthidazole was a strong and rapid inhibitor of photosynthesis of the sensitive redroot pigweed and quackgrass plants, with less effect on corn and alfalfa. Corn appeared to be tolerant to low rates of buthidazole following preemergence application. Postemergence application of buthidazole at rates as low as 0.28 kg/ha inhibited total photosynthesis of corn as early as 4 hours after spraying. Inhibition of anthocyanin formation in corn did not appear to be connected to the effect of this herbicide on photosynthesis since low rates

that inhibited anthocyanin biosynthesis did not inhibit total photosynthesis of corn following preemergence application of buthidazole. Buthidazole did not affect respiration of corn and redroot pigweed seedlings except for a transitory increase in corn 12 days after preemergence or 4 hours after postemergence treatment with buthidazole at 0.56 or 0.84 and 1.12 kg/ha, respectively. Respiration in alfalfa was also showed a transitory increase 4 hours after treatment with buthidazole at 1.12 and 2.24 kg/ha following postemergence application. A long-term inhibition of quackgrass respiration 96 hours after treatment with buthidazole at 1.12 and 2.24 kg/ha was evident.

Transmission electron microscopy studies showed that postemergence application of buthidazole at 0.28 and 1.12 kg/ha reduced or prevented starch accumulation in corn bundle sheath chloroplasts of treated plants as early as 4 hours after treatment, indicating thus interference with photosynthesis. Ultrastructural disruptions in some mesophyll chloroplasts of treated corn plants were also evident.

Germination studies with seeds of corn, redroot pigweed, alfalfa, and quackgrass indicated that buthidazole is not a germination inhibitor.

The mode of action studies with buthidazole indicate that a differential mode of buthidazole action between tolerant and susceptible species did not appear to contribute to crop selectivity.

Attempts to increase corn tolerance to higher rates of buthidazole by using herbicide antidotes found that CDAA (2-chloro-N,N-diallylacetamide) and NA (1,8-naphthalic anhydride) offered partial protection, with R-25788 (2,2-dichloro-N,N-diallylacetamide) being ineffective. CDAA appeared to be more effective than NA and a ratio 1:3 (buthidazole:CDAA) was optimal for the protection effect. Although this ratio, 1:3, may not be practical, it showed that buthidazole safety to corn could be chemically enhanced.

Στή μνήμη τοῦ πατέρα μου Κλεάνθη, τῆς μητέρας μου Διαμάντως,

καί τοῦ ἀδελφοῦ μου Πέτρου

In memory of my father Kleanthis, my mother Diamando, and

my brother Petros

Λίγο άκόμα

θά δοῦμε τίς άμυγδαλιές ν' άνθίζουν

τά μάρμαρα νά λάμπουν στόν ἥλιο

τή θάλασσα νά κυματίζει

Λίγο άκόμα,

νά σηκωθοῦμε λίγο ψηλότερα

Γιῶργος Σεφέρης , άπό τό Μυθιστόρημα

Just a little more

and we shall see the almond trees in blossom

the marbles shining in the sun

the sea, the curling waves

Just a little more

let us rise just a little higher

George Seferis, From Mythistorema

Greek poet, Nobel laureate, 1963

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CHAPTER 8

INTRODUCTION

Extensive use of a relatively persistent herbicide like atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] applied repeatedly to the same area for many years may create conditions for ecological population expansion of resistant strains of weed species. Biotypes of common groundsel (Senecio vulgaris L.), redroot pigweed (Amaranthus retroflexus L.), and common lambsquarters (Chenopodium album L.) resistant to atrazine have been reported (10,71,73). In a continuous corn (Zea mays L.) cropping system, rotation of herbicides applied from year to year may provide an effective means of preventing an ecological buildup of resistant strains. Therefore, apart from the economical reasons, development of new selective herbicides for weed control in corn is still a challenge to the herbicide industry.

Thiadiazole derivatives are chemical compounds containing the 1,3,4-thiadiazolyl ring and they have been reported to possess phytotoxicity (49,75). Original work in Japan revealed that 1,1-dimethyl-3-(5-tert-butyl-1,3,4-thiadiazol-2-yl)urea showed the strongest herbicidal activity among the thiadiazolyl urea derivatives tested (49). Later on, tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N-dimethylurea) and buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) were synthesized and developed as industrial herbicides in United States (2,3). These two herbicides have also shown promise for agricultural uses. Thus, buthidazole has shown potential for selective weed control in corn following preemergence or early postemergence application at low rates and in established alfalfa (Medicago sativa L.) applied in fall or spring. Among the plant species susceptible to buthidazole are redroot pigweed and quackgrass [Agropyron repens (L.) Beauv.], serious weed problems interfering with the production of corn and alfalfa, respectively. The reason for this selectivity of buthidazole between corn and redroot pigweed and between

alfalfa and quackgrass is not known. Differential rate of metabolism by N-demethylation has been proposed as the main factor contributing to selectivity of other thiadiazolyl urea herbicides between barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.) (57). However, differential absorption, translocation, and mode of action have also been recognized as bases for selective chemical weed control (9,27).

The margin of buthidazole selectivity between tolerant and susceptible plant species is very narrow. Use of herbicide antidotes or protectants offers a potential alternative for increasing the margin of selectivity (11, 68). Three compounds have received commercial interest as herbicide antidotes. These are NA (1,8-naphthalic anhydride), R-25788 (2,2-dichloro-N,N-diallylacetamide) and concep or CGA-43089 [α-(cyanomethoximino)-benzonitrile]. However, the concept of using herbicide antidotes has been successful only in protecting grass species, primarily corn, against injury from specific herbicidal groups such as the thiocarbamates and the acetanilides. Protection of broadleaf crop plants against injury from any herbicidal group or protection of any plant species against injury from herbicides known to act as photosynthetic inhibitors, like the triazines and the substituted ureas, has been unsuccessful with chemical compounds evaluated as herbicidal antidotes (43). However, antagonistic interactions leading to protection of broadleaf species from herbicide injury or antidoting the effect of photosynthetic inhibitors with other herbicides have been reported (30,51).

The objectives of this research were: a) to understand the basis for buthidazole selectivity by studying herbicide absorption, translocation, metabolism, and mode of action in corn, redroot pigweed, alfalfa, and quackgrass and b) to broaden the margin of buthidazole selectivity in corn by using antidotes or antagonistic interactions with other herbicides. In some of the mode of action studies the effects of buthidazole on plant physiological processes were compared to those of its analog tebuthiuron.

CHAPTER 1

Absorption, Translocation, and Metabolism of ^{14}C-Buthidazole in Alfalfa (Medicago sativa L.) and Quackgrass [Agropyron repens (L.) Beauv.]

ABSTRACT

The pattern of buthidazole (3-[5-(1,1dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) uptake, translocation, and metabolism and their potential contribution to crop selectivity were studied in tolerant alfalfa (Medicago sativa L.) and susceptible quackgrass [Agropyron repens (L.) Beauv.]. ^{14}C-buthidazole at 0.1 µCi was applied to a single leaf of established alfalfa and quackgrass plants and the plants harvested 1, 3, and 14 days after application. In the root uptake study, plants were placed for one day in Hoagland's no. 1 solution containing 10 µCi/L of ^{14}C-buthidazole and were harvested 1, 3, and 6 days after treatment. ^{14}C-buthidazole was absorbed by leaves of both alfalfa and quackgrass plants but did not move basipetally to the roots or nontreated leaves. ^{14}C-buthidazole was taken up very rapidly by the roots of both species and translocated to the leaves through the xylem. No differences in absorption and tranlocation of buthidazole between alfalfa and quackgrass were evident, indicating that these processes did not contribute to crop selectivity. Apart from unmetabolized ^{14}C-buthidazole, five metabolites were present in alfalfa leaf extracts 6 days after root treatment. A major unidentified metabolite had an Rf value of 0.21 (developing system; Chloroform 80%: Methanol 20%), and accounted for 40% of the detected radioactivity 6 days after root application. Metabolism of buthidazole was very slow in quackgrass. Unmetabolized ^{14}C-buthidazole was present as 87% of the total radioactivity even after 6 days. Two metabolites with Rf values similar to those of the amine and dihydroxy derivatives of buthidazole were present in

higher amounts in alfalfa as compared to quackgrass, whereas a metabolite similar to the methyl urea derivative of buthidazole was present only in alfalfa. Thus, differential rate of metabolism appeared to be a major factor contibuting to buthidazole selectivity between alfalfa and quackgrass.

INTRODUCTION

Buthidazole, marketed under the trade name Ravage^{TM}, is a relatively new herbicide used for industrial and non-cropland weed control (1). Use of buthidazole as selective herbicide for weed control in established alfalfa is under investigation (2). Buthidazole applied in spring of fall at rates 0.28 to 4.48 kg/ha has been found promising in controlling quackgrass, a tough perennial weed interfering with alfalfa production (2). The basis for this selectivity of buthidazole between alfalfa and quackgrass is not known.

Differential absorption, translocation, and metabolism have long been recognized as bases for selective chemical weed control (3,8). Most of the work on uptake, translocation, and metabolism of herbicides has been done with radioactive labeled compounds (5,7).

The purpose of this study was to determine the contribution of foliar and root uptake and translocation, and of metabolism following root uptake to buthidazole selectivity between alfalfa and quackgrass plants.

MATERIALS AND METHODS

Plant material. Seeds of 'Vernal' alfalfa were planted into wooden boxes 54 by 36 by 18 cm filled with a volume mixture of soil, sand, and peat (1:1:1). The plants were grown to maturity under greenhouse conditions of 33 C day and 20 C night temperature without additional artificial light. At flowering the plants were cut to 6 cm height, allowed to regrow to 12 cm, and placed outdoors to undergo dormancy during winter of 1978. The

plants were then transferred back to the greenhouse for acclimation, thinned to one plant per 946-ml cup, and when they reached 15 to 20 cm in height, they were used for the foliar application study. Alfalfa seedlings grown under greenhouse conditions to a height of 8 to 10 cm, were used for the root application study. Quackgrass plants were produced from rhizomes collected after dormancy from a field in East Lansing, Michigan. The rhizomes were placed in 946-ml waxed cups and grown under the same environmental conditions described earlier. When they reached the height of 18 to 25 cm, the plants were used in both the foliar and root application studies.

Foliar and root application of ^{14}C-buthidazole. A 5 µl drop containing 0.1 µCi of ^{14}C-buthidazole, labeled at the 5 carbon atom of the thiadiazole ring (Figure 1), was placed inside a lanolin ring enclosure on the second oldest leaf of quackgrass and in the middle leaf of one of the upper trifoliolate leaves of alfalfa. The specific activity of ^{14}C-buthidazole was 12.7 mCi/mmole. Following treatment, the plants were placed in a greenhouse with the same conditions as described earlier. Treated plants were harvested 1, 3, and 14 days after foliar application of ^{14}C-buthidazole.

For the root uptake study, alfalfa and quackgrass plants were placed into modified Hoagland's no. 1 solution (6) containing 10 µCi/L of ^{14}C-buthidazole for one day and then transferred to non-radioactive Hoagland's no. 1 solution. The growing conditions were the same as those of the leaf application study. The plants were harvested 1, 3, and 6 days after root application.

Following both the foliar and root applications of ^{14}C-buthidazole, the harvested plants were divided into treated leaf, remaining shoot, and roots. Radioactivity was determined by combustion and is expressed as dpm/mg of leaf or root dry tissue. Translocation was determined both qualitatively by radioautography and quantitatively by radioassay of the various

portions of the treated plants. For the radioautography, the harvested plants were freeze-dried, rehydrated, mounted on blotter paper, pressed, and radioautographed. Figures are representative of two experiments with two replications per each experiment. For the quantitative measurement of translocation, harvested plants were freeze-dried and dissected into various sections according to the type of buthidazole application. Thus, in the foliar application, plants were sectioned into the treated leaf, shoot and non-treated leaves, and roots. In the roots application, the plants were divided into shoots and leaves and into the roots. These plant portions were homogenized in an Sorval-Omni mixer for 5 minutes in 20 ml of 100% methanol, and each homogenate was filtered through Whatman #1 filter paper under vacuum. Aliquots of 0.5 ml from the methanol-soluble extracts were used for radioactivity determinations by liquid scintillation spectrometry. The methanol-insoluble residues were combusted under O_2 in a biological oxidizer (OX-200; P. J. Harvey Instr.) and their radioactivity was again determined by liquid scintillation radioassay (Beckman LS 8100). The results of the radioactivity measurements were expressed as percent (%) of the total ^{14}C found and as dpm/mg of plant tissue. Data presented are the means of two experiments with two replications per experiment. Percent values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance. Duncan's multiple range test was used for mean separation.

Extraction, Separation, and Quantitation of Buthidazole and its Metabolites. Since uptake by roots and rapid translocation to the shoots and leaves appeared to be the predominant pattern of buthidazole in the previous studies with both plant species, the methanol-soluble extracts from the shoots and leaves of the root-treated plants, obtained as described in the translocation study, were used for the metabolism studies. Partitioning of 15 ml of the

methanol-soluble leaf extracts against 15 ml of hexane still left 95% of the radioactivity in the aqueous fraction. One hundred µl samples from the aqueous phase of each extract were spotted on Thin Layer Chromatography (TLC) plates precoated with silica gel G layer 250 µm thick. The plates were developed in a solvent system containing 80% chloroform and 20% methanol (4:1), and the Rf values were determined following application of radioautography and TLC-scanning with a Berthold LB 76 scanner with a slit width 2 mm. Quantitation of the radioactivity present in each metabolite was done by integration of the peaks obtained for each spot using the TLC scanner. The results are expressed as percent of the total radioactivity found in the 100 µl sample of each extract used.

Identification of Metabolites. The TLC absorbant containing the ^{14}C labeled methanol-extractable spots was removed from the plate, extracted with 2 ml of methanol, centrifuged to 500 x g, and filtered through a glass fiber filter under vacuum. The filtrates were evaporated down to 100 µl under nitrogen. Then the separated metabolites were co-chromatographed with known compounds on 20 by 20 cm plates (Redi/Plate, Analtech, Inc.) coated with silica gel GF (250 µm) in the same solvent system of chloroform:methanol (4:1). Localization of the metabolites was achieved by exposure to ultraviolet light (UV lamp 254 nm). Presence of small amounts of unknown ^{14}C-metabolites was confirmed by TLC scanning. The following buthidazole derivatives used for identification were kindly provided by Velsicol Chemical Corporation: a) Analytical grade buthidazole 98.7% pure by Infrared Spectroscopy (IR). b) Buthidazole-DiOH (3-[5-(1,1dimethylethyl)-1,3,4-thiadiazol-2-yl]-4,5-dihydroxy-2-imidazolidinone), 100% pure by TLC. c) Buthidazole methylurea (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-1-methyl)urea, 99% pure by TLC. d) Buthidazole urea (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-urea), 99% pure by TLC and e) Buthidazole

amine [5-amino-2-(1,1-dimethylethyl)-1,3,4-thiadiazole], 99.8% pure by Liquid chromatography.

RESULTS AND DISCUSSION

Following foliar ^{14}C-buthidazole application, alfalfa and quackgrass absorbed almost equal amounts of ^{14}C-buthidazole expressed either as dpm/plant or dpm/mg of dry treated leaf tissue (Table 1). When ^{14}C-buthidazole was supplied to the roots, quackgrass absorbed five times more herbicide than alfalfa seedlings when the radioactivity was expressed as dpm/plant (Table 2). However, when the results were expressed as dpm/mg of leaves or roots, alfalfa seedlings concentrated two times more ^{14}C-buthidazole in the leaves and seven times as much ^{14}C-buthidazole in the roots as quackgrass did (Table 2). Therefore, the observed results suggest that the plant size was the reason for the differences in absorption since alflafa plants used were seedlings while the quackgrass used were older plants. Furthermore, these results show that alfalfa seedlings rapidly take up and concentrate buthidazole. The difference in depth of rooting between established alfalfa and quackgrass may also contribute to selectivity of buthidazole under field conditions.

Buthidazole did not translocate appreciably in both alfalfa and quackgrass plants following foliar application at any harvesting period after the treatment (Figures 2 and 3). Acropetal movement toward the tip of the treated leaf was evident but no signs of basipetal translocation were present. Radioassay of various plant parts of the treated plants revealed the same pattern of acropetal movement in both plant species (Tables 3 and 4).

Rapid translocation of ^{14}C-buthidazole from the roots to the leaves of both alfalfa and quackgrass plants was evident in the radioautographs shown in Figures 4 and 5. Quantitative measurement of translocation indicated that 1 day after root application of buthidazole 90 and 66% of the

radioactivity detected in both the methanol-soluble and insoluble parts was translocated to the leaves of alfalfa and quackgrass, respectively (Tables 5 and 6). Examining the results obtained from both the foliar and root application studies, it appears that rapid uptake by the roots and rapid translocation to the leaves where the herbicide exerts its action by inhibiting photosynthesis (4) seems to be the pattern of uptake and translocation of buthidazole with little difference between species.

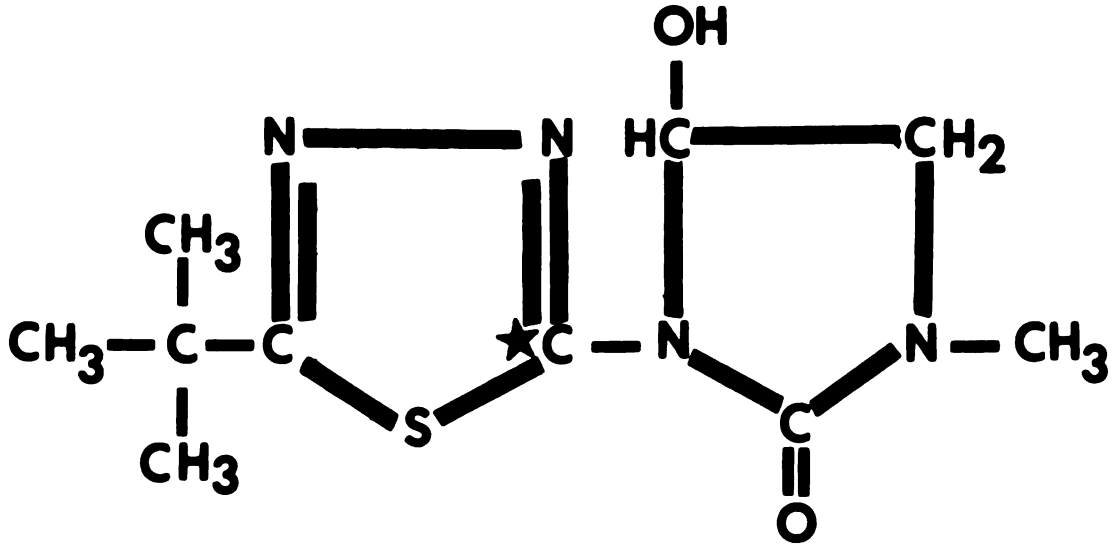
One day after root application alfalfa metabolized ^{14}C-buthidazole very rapidly, yielding at least five metabolites apart from the unmetabolized parent compound (Table 7). The distribution of the radioactivity detected in each metabolite revealed that an unidentified metabolite with Rf value of 0.21 seems to be the major metabolite in alfalfa with the others being minor. Data presented in Table 7 indicates that the amount of radioactivity associated with this metabolite increased as a function of time, whereas the radioactivity in the parent compound decreased along with time. For comparison reasons, the Rf values of the analytical reference buthidazole derivatives used for identification are included in Tables 7 and 8, below the unknown metabolites with Rf values similar to the respective standard. Thus, metabolites #3, 4, and 5 with Rf values of 0.51, 0.60, and 0.64, respectively, appeared to be similar to the amine, dihydroxy, and methylurea derivatives of buthidazole (Table 7). One more unidentified metabolite with Rf value of 0.42 accounted for 17% of the total radioactivity after 6 days (Table 7).

Quackgrass does not metabolize ^{14}C-buthidazole rapidly (Table 8). Unmetabolized ^{14}C-buthidazole was present as 87% of the total radioactivity even after 6 days. An unknown metabolite with Rf value of 0.25, believed to be similar to the major metabolite found in alfalfa, was present only in small amounts (Table 8). Two minor metabolites with Rf values resembling those

of the amine and dihydroxy derivatives of buthidazole were also present in quackgrass but in smaller amounts than in alfalfa (Tables 7 and 8). It is clear, therefore, from the data obtained in the metabolism studies that metabolism appears to be a major factor contributing to buthidazole selectivity between alfalfa and quackgrass. The results of buthidazole metabolism in alfalfa seem to be in agreement with those reported elsewhere (9). Buthidazole metabolites in alfalfa previously reported included 5-amino-(1,1-dimethylethyl)-1,3,4-thiadiazole, 3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-hydroxy-2-imidazolidinone and 5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl)urea (9). Whether the major unidentified metabolite in alfalfa resembles the desmethyl derivative, which has been reported as a major metabolite in the aforementioned study (9), or another unknown buthidazole derivative is not known yet.

In summary then , we conclude that buthidazole is absorbed by the leaves and roots of both alfalfa and quackgrass in a similar manner. Translocation followed the apoplastic pathway in the xylem driven by the transpiration stream in both alfalfa and quackgrass plants. Rapid metabolism of buthidazole by alfalfa seedlings appears to be the basis for the observed selectivity of this herbicide between alfalfa and quackgrass. An unidentified metabolite with Rf value of 0.21 accounted for 40% of the total radioactivity in alfalfa leaves 6 days after root application of ^{14}C-buthidazole and is believed to be very important for the observed selectivity. Quackgrass only slightly metabolized buthidazole.

Figure 1. Chemical structure and chemical name of the herbicide buthidazole. The asterisk (*) indicates the radioactive labeled carbon atom (^{14}C).



3-[5-(1,1-dimethylethyl)-1,3,4- thiadiazol-2-yl]-4- hydroxy-1-methyl-2- imidazolidinone

BUTHIDAZOLE

Figure 2. Translocation of ^{14}C-buthidazole in alfalfa plants. Treated plants harvested (A) 1 day, (B) 3 days, and (C) 14 days after foliar application. Corresponding radioautographs below (a,b,c).

Figure 3. Translocation of ^{14}C-buthidazole in quackgrass plants. Treated plants above harvested (A) 1 day, (B) 3 days and (C) 14 days after foliar application. Corresponding radioautographs below (a,b,c).

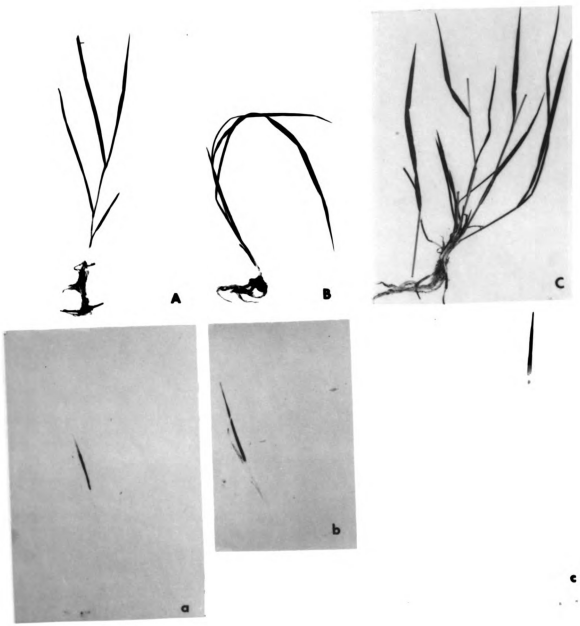

A
B
C
a
b
c

Figure 4. Translocation of ^{14}C-buthidazole in alfalfa plants. Treated plants above harvested (A) 1 day, (B) 3 days, and (C) 6 days after root application. Corresponding radioautographs below (a,b, c).

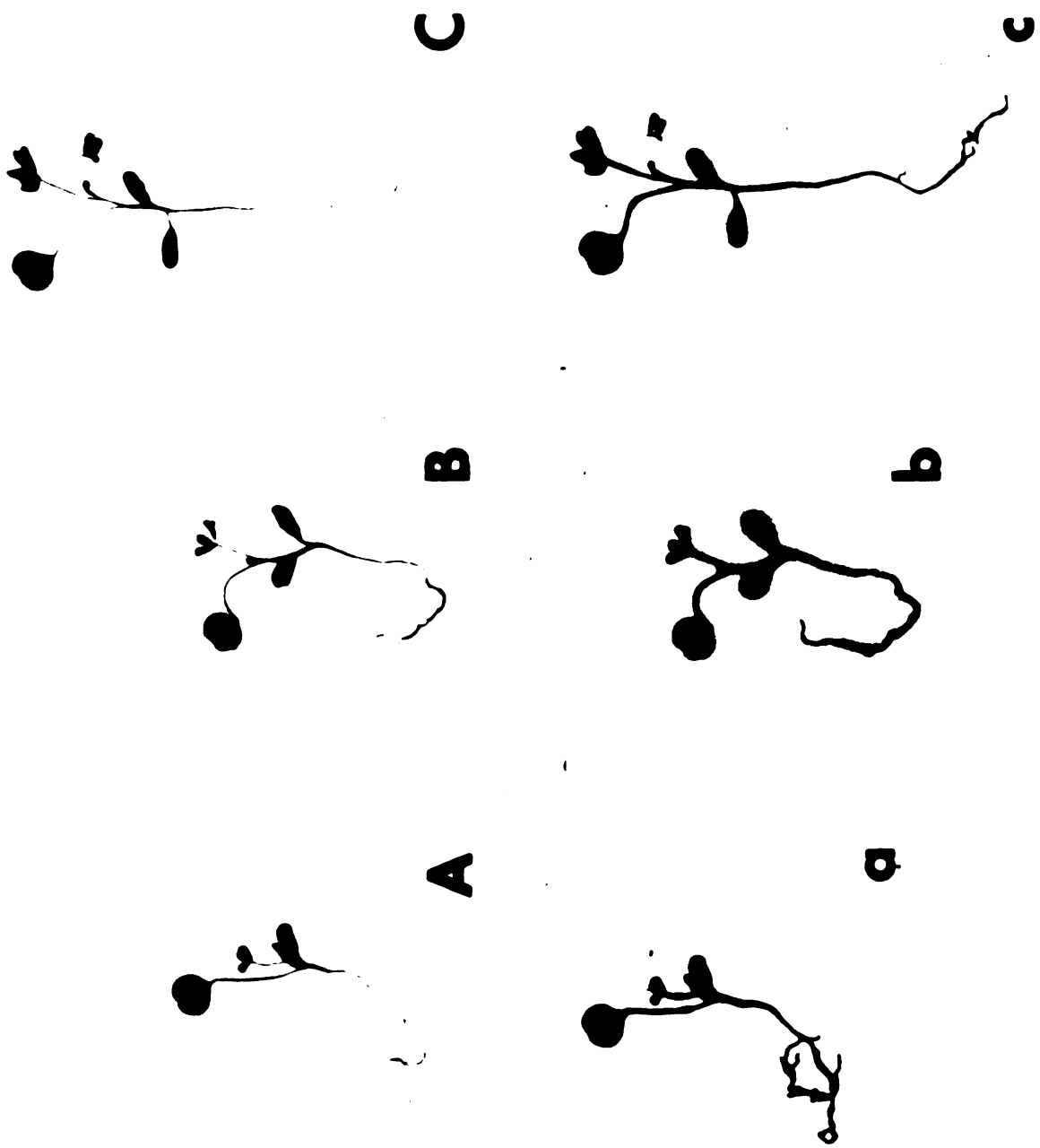

C
c
B
b
A
a

Figure 5. Translocation of ^{14}C-buthidazole in quackgrass plants. Treated plants above harvested (A) 1 day, (B) 3 days, and (C) 6 days after root application. Corresponding radioautographs below (a, b, c).

Table 1. ^{14}C-Buthidazole absorption by alfalfa and quackgrass plants harvested 1, 3, and 14 days after leaf treatment[a].

Species	Harvesting Time		^{14}C-uptake	
			Plant	Treated leaf
	(days)		(dpm/plant)	(dpm/mg)
Alfalfa	1		32563 a	5617 a
	3		17980 a	4448 a
	14		16875 a	2954 a
		Mean	22473	4340
Quackgrass	1		31937 a	4916 a
	3		31915 a	2787 a
	14		27652 a	3586 a
		Mean	30501	3763

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. ^{14}C-Buthidazole absorption by alfalfa and quackgrass plants harvested 1, 3, and 6 days after root application[a].

Species	Harvesting Time	^{14}C-uptake		
		Plant	leaves	roots
	(days)	(dpm/plant)	(dpm/mg)	(dpm/mg)
Alfalfa	1	36787 a	15183 bc	5066 b
	3	37597 a	20567 c	2213 a
	6	37699 a	19054 c	1331 a
	Mean	37361	18268	2870
Quackgrass	1	184124 c	6352 a	852 a
	3	112352 b	11189 ab	283 a
	6	157482 bc	5726 a	262 a
	Mean	151319	7756	466

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. Distribution of total ^{14}C found in various parts of alfalfa plants harvested at 1,3, and 14 days after leaf application of ^{14}C-buthidazole[a].

Plant part	1 day		3 days		14 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol-soluble						
Treated leaf	91.9 b	5526.9 c	92.5 b	4423.1 c	90.6 b	2917.5 b
Shoots and leaves	4.6 a	40.6 a	4.1 a	21.5 a	3.7 a	3.3 a
Roots	1.8 a	7.2 a	1.3 a	2.5 a	1.9 a	1.8 a
Methanol-insoluble						
Treated leaf	1.3 a	90.3 a	1.0 a	24.9 a	1.3 a	36.7 a
Shoots and leaves	0.2 a	2.3 a	0.6 a	1.7 a	1.5 a	0.7 a
Roots	0.2 a	0.7 a	0.5 a	0.6 a	1.0 a	0.7 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 4. Distribution of total ^{14}C found in various parts of quackgrass plants harvested at 1, 3, and 14 days after leaf application of ^{14}C-buthidazole[a].

Plant part	1 day		3 days		14 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol-soluble						
Treated leaf	94.6 b	4881.9 c	90.0 b	2757.7 b	94.7 c	3525.9 bc
Shoot and leaves	2.8 a	17.5 a	5.2 a	17.0 a	1.8 a	3.5 a
Roots and rhizomes	1.3 a	4.3 a	1.9 a	4.2 a	0.7 a	0.8 a
Methanol-insoluble						
Treated leaf	0.9 a	33.9 a	1.9 a	29.6 a	1.8 a	59.8 a
Shoot and leaves	0.2 a	1.1 a	0.5 a	0.9 a	0.5 a	0.5 a
Roots and rhizomes	0.2 a	0.7 a	0.5 a	0.7 a	0.5 a	0.3 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 5. Distribution of total ^{14}C found in various parts of alfalfa plants harvested at 1, 3, and 6 days after root application of ^{14}C-buthidazole[a].

Plant part	1 day		3 days		6 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol-soluble						
Shoots and leaves	89.0 c	16303.7 b	93.7 d	19996.0 b	93.9 d	18459.0 b
Roots	6.7 b	3931.6 a	2.0 a	1176.0 a	1.4 a	699.6 a
Methanol-insoluble						
Shoots and leaves	2.2 a	397.8 a	3.0 ab	571.3 a	3.3 a	594.7 a
Roots	2.1 a	1134.2 a	1.3 a	1036.9 a	1.4 a	631.2 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 6. Distribution of total ^{14}C found in various parts of quackgrass plants harvested at 1, 3, and 6 days after root application of ^{14}C-buthidazole[a].

Plant part	1 day		3 days		6 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol-soluble						
Shoot and leaves	59.2 c	5716.0 b	89.0 e	10966.8 c	83.0 d	5181.0 b
Roots and rhizomes	26.0 b	610.2 a	4.2 a	130.7	7.3 a	207.9 a
Methanol-insoluble						
Shoot and leaves	6.8 a	636.3 a	2.1 a	222.7 a	8.0 a	544.6 a
Roots and rhizomes	8.0 a	240.8 a	4.7 a	121.1 a	1.7 a	53.7 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 7. Methanol-soluble metabolites of ^{14}C-buthidazole in alfalfa leaves and stems 1, 3, and 6 days after root application[a].

Metabolite	Rf value*	Days after treatment 1	3	6
		(% of total radioactivity)		
Unknown #1	0.21	13.2 de	31.5 g	39.8 i
Unknown #2	0.42	6.6 b	9.8 c	17.0 f
Unknown #3	0.51	2.4 a	16.0 ef	12.5 cd
Buthidazole amine	0.54			
Unknown #4	0.60	4.7 ab	3.8 ab	13.6 de
Buthidazole DiOH	0.62			
Unknown #5	0.64	1.9 a	1.9 a	2.3 a
Buthidazole methyl urea	0.65			
Buthidazole	0.71	71.2 j	37.0 h	14.8 def

* Developing system; Chloroform : Methanol (4:1).

[a] Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 8. Methanol-soluble metabolites of ^{14}C-buthidazole in quackgrass leaves and stems 1, 3, and 6 days after root application[a].

Metabolite	Rf value*	Days after treatment 1	3	6
		(% of total radioactivity)		
Unknown #1	0.25	1.9 a	4.8 a	6.5 a
Unknown #2	0.50	1.5 a	2.5 a	1.9 a
Buthidazole amine	0.54			
Unknown #3	0.61	6.1 a	5.6 a	4.8 a
Buthidazole DiOH	0.62			
Buthidazole	0.73	90.5 b	87.1 b	86.8 b

* Developing system; Chloroform : Methanol (4:1)

[a] Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

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CHAPTER 2

Site of Uptake and Translocation of ^{14}C-Buthidazole in Corn (*Zea mays* L.) and Redroot Pigweed (*Amaranthus retroflexus* L.)

ABSTRACT

Uptake and translocation of ^{14}C-buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) in corn (*Zea mays* L.) and redroot pigweed (*Amaranthus retroflexus* L.) were studied following both foliar and root treatments under greenhouse and growth chamber environments. Following foliar application, ^{14}C-buthidazole was absorbed by the leaves of corn and redroot pigweed seedlings in similar amounts. Translocation occured only toward the tip of the treated leaves in corn, whereas in redroot pigweed the ^{14}C moved both acropetally and basipetally. Rapid uptake by the roots and rapid movement to the leaves via the xylem seems to be the main pathway of uptake and translocation of ^{14}C-buthidazole supplied to the roots of redroot pigweed plants. Uptake by both the roots and the emerging coleoptile and transport to the foliage seems to be the pattern of absorption and translocation of buthidazole in corn following preemergence application. Difference in absorption did not appear to be an important factor contributing to selectivity of ^{14}C-buthidazole between corn and redroot pigweed. However, translocation of ^{14}C-buthidazole supplied to the roots was faster to the redroot pigweed shoots than to corn shoots.

INTRODUCTION

Extensive use of a relatively persistent herbicide like atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] applied repeatedly to the same area for many years may create conditions for the growth of resistant strains of weed species. Thus, common groundsel (*Senecio vulgaris* L.), redroot pigweed, and common lambsquarters (*Chenopodium album* L.) have been reported to have strains resistant to atrazine (3,7,8). In a continuous corn cropping system, rotation of herbicides applied from year to year may

provide an effective means of preventing an ecological buildup of resistant strains. Therefore, apart from the economical reasons, development of new selective herbicides for weed control in corn is still a challenge to the herbicide industry.

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1- methyl-2-imidazolidinone) is a new promissing herbicide for selective weed control in corn applied preemergence or early postemergence (1). For herbicides applied preemergence or preplant incorporated, the roots and emerging coleoptile of grass species are important pathways of herbicide entry. In postemergence-applied herbicides, the leaves and occasionally the roots are important routes of entry of the herbicide.

The objectives of this study were a) to examine ^{14}C-buthidazole absorption and translocation in tolerant corn and susceptible redroot pigweed following foliar and root applications, b) to determine the site of buthidazole uptake in corn following preemergence application and c) to determine whether differential absorption or translocation of buthidazole plays a role in crop selectivity.

MATERIALS AND METHODS

Foliar and Root Applications of ^{14}C-buthidazole.

a. Plant material. Five 'Pioneer 3780' corn and ten redroot pigweed seeds were planted 4.0 and 0.5 cm deep, respectively, into greenhouse soil (1:1:1 soil, sand, peat) in 946-ml waxed cups. After planting, the cups were placed in a greenhouse with temperature ranging from 20 C at night to 33 C during the day without additional artificial light. After emergence, the plants were thinned to one plant per cup. Corn seedlings 15 cm tall and redroot pigweed seedlings 10 cm tall were used for the uptake and translocation studies.

b. Application of ^{14}C-buthidazole. Radioactive buthidazole was labeled at the 5 carbon atom of the thiadiazole ring and had a specific activity

of 12.7 mCi/mM. A 5 µl drop containing 0.1 µCi of ^{14}C-buthidazole was placed on the center area of the second older leaf of corn and redroot pigweed seedlings. Following treatment, the plants were moved to a growth chamber with a 16-h day and a light intensity of 19 Klux. Day and night temperature was maintained at 25 ± 1 C. Treated plants were harvested 1, 3, and 14 days after treatment. In the root uptake study the roots of corn and redroot pigweed seedlings were placed in a modified Hoagland's (6) No. 1 solution containing 10 µCi/L of ^{14}C-buthidazole. The plants remained in the radioactive Hoagland's solution for only 1 day. Then they were transferred and continued to grow in cups containing Hoagland's No. 1 solution without ^{14}C-buthidazole. The environmental conditions throughout this study were the same as those in the foliar application. The plants were harvested after 1, 3, and 6 days for redroot pigweed and 1, 3, and 14 days for corn. The susceptible redroot pigweed plants were near death 6 days after treatment.

Following both foliar and root applications of ^{14}C-buthidazole, the treated plants were analyzed for radioactivity in the specific plant portions. Radioactivity measurements were expressed as dpm/plant or dpm/mg of leaf or root tissue. Translocation was determined both qualitatively by radioautography and quantitatively by radioassay with liquid scintillation spectrometry of the radioactivity found in various parts of the treated plants following combustion of the plant parts. The treated plants were divided into treated leaf, shoot and non-treated leaves, and roots following the foliar application and into shoot and leaves and roots following the root treatment. Photographs presented are representative of two experiments each with two replications. Radioactivity determinations are expressed as percent (%) of the total or they are calculated as dpm/mg of ^{14}C-buthidazole. Data presented are the means of two experiments each with two replications. Percent data involving values less than 15% or greater than 85% were

transformed to arcsine values for analysis of variance. Duncan's multiple range test was used for mean separation.

Site of Buthidazole Uptake Following Preemergence Application to Corn.

Determination of the site of buthidazole uptake by corn included three separate studies. First, the effect of buthidazole on corn growth was examined after selective application of the herbicide to various corn root and shoot regions. Comparison of corn injury caused by buthidazole, applied either preemergence or preplant incorporated into the soil, was the subject of the second study. Finally, placement of ^{14}C-buthidazole on the emerging coleoptile of corn seedlings was used in the third study to determine the contribution of absorption by the coleoptile in buthidazole uptake by corn.

In the first study a slight modification of the method reported by Armstrong et al. (2) was used. An activated charcoal layer separated the root and shoot regions of corn and prevented vertical movement of the herbicide in the soil. Buthidazole was applied preemergence separately to the root zone (below the charcoal layer) and on the soil surface at rates of 0, 0.56, 1.12, and 2.24 kg/ha. Five 'Pioneer 3780' corn seeds were planted into greenhouse soil in 946-ml waxed cups. After planting and treatment with buthidazole, the cups were placed in a greenhouse with the same conditions as in the foliar and root uptake studies. Water was applied to provide moisture for growth of the plants. Shoot height and fresh weight were recorded 30 days after planting and treatment with the herbicide.

In the second study buthidazole was applied either preemergence on the soil surface or preplant incorporated into the top 6.0 cm of soil at rates of 0, 0.56, 1.12, and 2.24 kg/ha. Five 'Pioneer 3780' corn seeds were planted 5.0 cm deep and the cups were placed in the same greenhouse as in the previous study. Thirty days after treatment, shoot height and shoot fresh weight were recorded. In both the first and second studies

two experiments each with two replications were performed and the data analyzed for variance followed by Duncan's multiple range test.

In the last study a 5 µl drop containing 0.2 µCi of ^{14}C-buthidazole was placed on the emerging coleoptile of 'Pioneer 3780' corn seedlings as soon as they penetrated the soil surface. Following treatment, the plants were placed in a growth chamber with the same environmental conditions as those described in the foliar and root application studies. Treated plants were harvested at 3, 8, and 16 days after treatment, radioautographed, and analyzed for translocation by radioassay after dissection into four parts. These parts were the first leaf, shoot and untreated leaves, the primary roots, and the adventitious roots. Radioactivity determinations were again expressed as percent (%) of the total or dpm/mg of the plant part. Photographs and data presented are from two experiments with two replications per experiment.

RESULTS AND DISCUSSION

Three days following foliar application, corn and redroot pigweed had absorbed similar amounts of radioactivity, expressed as dpm/plant (Table 1). However, when the results are expressed as dpm/mg in the treated leaf, pigweed absorbed more ^{14}C-buthidazole than did corn (Table 1). When ^{14}C-buthidazole was supplied to the root system, both corn and pigweed absorbed the same amount of radioactivity on a per plant basis (Table 2). Dividing the plant into leaves and roots and expressing the results as dpm/mg of leaf or root tissue indicates that pigweed concentrated more ^{14}C-buthidazole into the roots after 3 days than did corn (Table 2). These results indicate that there were no substantial differences in buthidazole uptake by the roots of the two plant species and suggest that the role of absorption by roots was not important for crop selectivity following preemergence application of buthidazole.

Translocation of ^{14}C-buthidazole in corn was limited to acropetal movement toward the tip of the treated leaf following foliar application (Figure 1). Quantitative measurement of translocation shows also that the bulk of radioactivity was present in the treated leaf even 14 days after application (Table 3). On the contrary, both acropetal and basipetal translocation of ^{14}C-buthidazole was evident in radioautographs from redroot pigweed plants (Figure 2). Quantitative measurements indicate that there were no differences in the distribution pattern of ^{14}C found in various plant parts as a function of time (Table 4). Thus distribution was rapid and occurred within 1 day. Fourteen days after treatment 65% of the total radioactivity was present in the treated leaf, 33% was translocated to the shoots and other leaves, and only traces were found in the roots (Table 4). Rapid uptake from the roots and translocation of ^{14}C-buthidazole to the leaves was observed to occur in both corn and redroot pigweed plants following root application of the herbicide (Figures 3 and 4). Therefore, movement of the herbicide from the roots to the leaves seems to follow primarily the apoplastic route, through the xylem in the transpiration stream. Furthermore, 14 days after treatment, the new leaves of corn plants formed after the treatment with the ^{14}C-labeled herbicide contained very little or insignificant amounts of radioactivity (Figure 3). Data presented in Tables 5 and 6 show that the percent of ^{14}C-buthidazole or of its metabolites increased in leaves and decreased in the roots as a function of time in corn (Table 5), whereas in the redroot pigweed plants 97% of the radioactivity moved to the leaves duing the first day after the treatment (Table 6). Thus, while the pattern of buthidazole uptake and translocation seems to be the same in corn and pigweed following root application, redroot pigweed seems to translocate the herbicide to the shoot faster than corn. These results are consistent with the observed selectivity.

Site of Buthidazole Uptake by Corn Following Preemergence Application.

Preemergence placement of buthidazole on the soil surface reduced both plant height and fresh weight of the new seedlings (Table 7). Placement of buthidazole in the root zone of corn (below the charcoal layer) did not affect corn seedlings even at rates up to 2.24 kg/ha. In grasses like corn, the primary root lives a relatively short time and the root system is formed by adventitious roots arising from the shoot (5). Therefore, buthidazole absorption by the adventitious roots could have been very important in the previous study. Buthidazole uptake by the emerging coleoptile of corn seedlings could also have been important in this study. However, since primary roots were found capable of absorbing buthidazole in the root application studies described earlier, inactivation of buthidazole by the activated charcoal layer appears to be the most logical explanation for the abscence of injury observed in corn seedlings following application of buthidazole to the root zone (Table 7). Incorporation of herbicides into the upper 6.0 to 10.0 cm of soil decreased the herbicide concentration at the soil surface, and one might expect decreased absorption by the emerging shoots. However, comparison of preemergence application versus preplant incorporation of buthidazole showed that preplant incorporated treatment was more active than the preemergence treatment (Table 8) indicating that uptake by both the primary and adventitious roots may be important. This supports the previous conclusion that buthidazole was inactivated by the activated charcoal layer used to separate the root and shoot zones. A study with acetanilide herbicides, which are absorbed primarily by newly emerging yellow nutsedge shoots, showed that preplant incorporated treatment provided greater control than the preemergence treatment (4). Information shown in Figure 5 and Table 9 indicates that uptake by the emerging coleoptile played a role in buthidazole absorption but the bulk of radioactivity, even 16 days after treatment,

remained in the first leaf with the remainder in the older leaves (Table 9).

In summary, then, buthidazole was absorbed by the leaves of both corn and redroot pigweed seedlings following foliar application but moved only acropetally in corn in contrast to redroot pigweed in which both acropetal and basipetal movement was observed. Rapid uptake by the roots and rapid movement to the leaves via the xylem occured in redroot pigweed. Uptake by both the roots and the emerging coleoptile of corn, followed by translocation to the leaves, was the pattern of buthidazole absorption and translocation in corn following preemergence application. Differences in absorption did not appear to be an important factor contributing to selectivity of buthidazole between corn and redroot pigweed. However, translocation of ^{14}C-buthidazole supplied to the roots was faster to the shoots of redroot pigweed than to corn shoots. Thus, faster translocation of buthidazole in redroot pigweed appears to be important for the observed selectivity.

Figure 1. Translocation of ^{14}C-buthidazole in corn plants. Treated plants on the left harvested (A) 1 day, (B) 3 days, and (C) 14 days after foliar application. Corresponding radioautographs to the right (a,b,c).


c
C
a
b
A
B

Figure 2. Translocation of ^{14}C-buthidazole in redroot pigweed plants. Treated plants on the left harvested (A) 1 day, (B) 3 days, and (C) 14 days after foliar application. Corresponding radioautographs on the right (a,b,c).

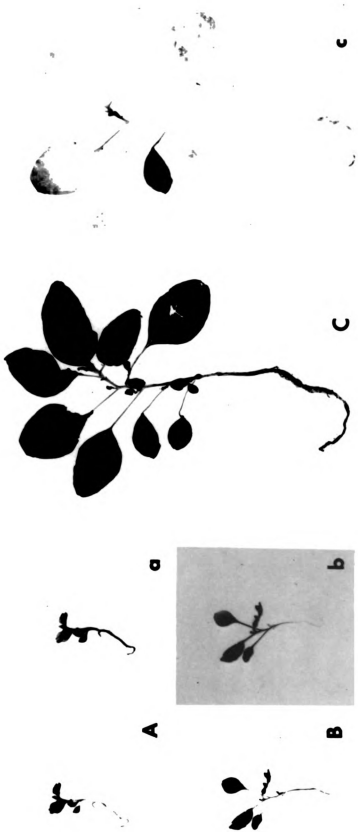
a
b
c
A
B
C

Figure 3. Translocation of ^{14}C-buthidazole in corn plants. Treated plants above harvested (A) 1 day, (B) 3 days, and (C) 14 days after root application. Corresponding radioautographs below (a,b,c).

Figure 4. Translocation of ^{14}C-buthidazole in redroot pigweed plants. Treated plants above harvested (A) 1 day, (B) 3 days, and (C) 6 days after root application. Corresponding radioautographs below (a,b,c).

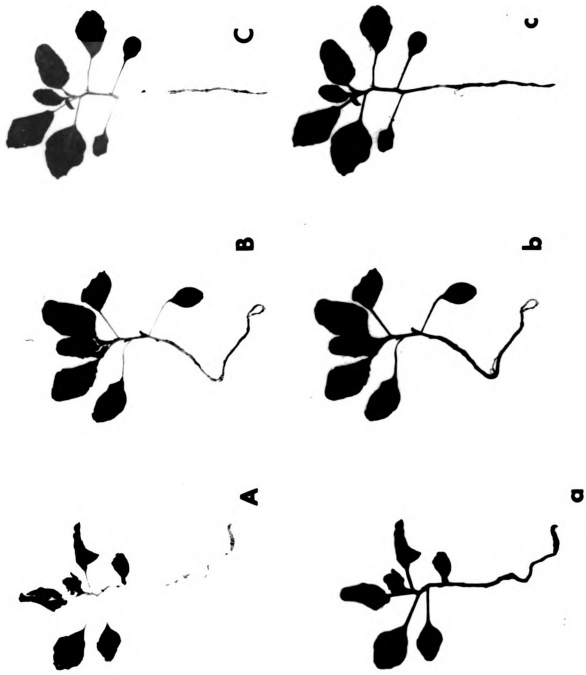
C
c
B
b
A
a

Figure 5. Translocation of ^{14}C-buthidazole in corn plants. Treated plants above harvested (A) 3 days, (B) 8 days, and (C) 16 days after application to the emerging coleoptile. Corresponding radioautographs below (a,b,c).

A
B
C
a
b
c

Table 1. ^{14}C-Buthidazole absorption by corn and redroot pigweed seedlings harvested 1, 3, and 14 days after leaf application[a].

Species	Harvesting time (days)		^{14}C uptake	
			Plant (dpm/plant)	Treated leaf (dpm/mg)
Corn	1		20079 ab	1316 a
	3		24077 b	663 a
	14		12720 a	397 a
		Mean	18959	792
Redroot pigweed	1		17191 ab	3091 b
	3		22824 b	3324 b
	14		23165 b	1235 a
		Mean	21060	2550

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. ^{14}C-Buthidazole absorption by corn and redroot pigweed seedlings harvested 1 and 3 days after root application[a].

Species	Harvesting time (days)		^{14}C uptake		
			Plant (dpm/plant)	Leaves (dpm/mg)	Roots (dpm/mg)
Corn	1		161395 a	6116 ab	1081 a
	3		194146 a	4513 a	855 a
		Mean	177770	5315	968
Redroot pigweed	1		195059 a	7089 ab	1655 ab
	3		177272 a	6984 ab	2373 b
			186165	7037	2014

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. Distribution of total ^{14}C found in various parts of corn plants harvested 1, 3, and 14 days after leaf application of ^{14}C-buthidazole.[a]

Plant part	1 day		3 days		14 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol - soluble						
Treated leaf	91.0 c	1273.5 d	90.0 c	619.7 c	83.4 b	343.5 b
Shoot and leaves	2.3 a	4.0 a	0.6 a	1.1 a	0.0 a	0.0 a
Roots	2.6 a	7.4 a	1.3 a	2.7 a	0.8 a	0.5 a
Methanol - insoluble						
Treated leaf	3.6 a	42.7 a	7.8 a	46.1 a	12.6 a	53.7 a
Shoot and leaves	0.3 a	0.5 a	0.2 a	0.4 a	2.6 a	0.8 a
Roots	0.2 a	0.5 a	0.1 a	0.3 a	0.6 a	0.3 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 4. Distribution of total ^{14}C found in various parts of redroot pigweed plants harvested 1, 3, and 14 days after leaf application of ^{14}C-buthidazole.[a]

	1 day		3 days		14 days	
Fraction plant part	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol - soluble						
Treated leaf	66.5 c	4058.0 d	55.5 c	3291.8 d	64.6 c	1216.6 bc
Shoots and leaves	29.1 b	2055.9 c	39.4 b	653.6 ab	27.9 b	30.6 a
Roots	2.8 a	601.3 ab	0.9 a	216.2 a	0.9 a	3.0 a
Methanol - insoluble						
Treated leaf	0.3 a	28.6 a	0.6 a	32.7 a	0.8 a	18.1 a
Shoots and leaves	1.1 a	23.8 a	3.5 a	84.5 a	5.6 a	3.1 a
Roots	0.2 a	26.2 a	0.1 a	25.9 a	0.2 a	0.4 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 5. Distribution of total ^{14}C found in various parts of corn plants harvested 1, 3, and 14 days after root application of ^{14}C-buthidazole.[a]

Plant part	1 day		3 days		14 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol - soluble						
Shoots and leaves	58.4 d	5967.1 c	80.0 e	4435.2 b	76.5 e	651.8 a
Roots	35.9 c	973.4 a	17.0 b	789.5 a	11.7 ab	195.0 a
Methanol - insoluble						
Shoots and leaves	1.4 a	148.7 a	1.5 a	78.2 a	10.2 ab	72.6 a
Roots	4.3 ab	107.4 a	1.5 a	65.5 a	1.6 a	32.2 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 6. Distribution of total ^{14}C found in various parts of redroot pigweed plants harvested 1, 3, and 6 days after root application of ^{14}C-buthidazole.[a]

Plant part	1 day		3 days		6 days	
	(% of total)	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol - soluble						
Shoots and leaves	94.2 b	5487.7 b	94.6 b	6860.5 bc	94.9 b	8496.2 c
Roots	2.2 a	1302.8 a	2.7 a	1933.9 a	1.9 a	781.2 a
Methanol - insoluble						
Shoots and leaves	3.0 a	1610.5 a	1.9 a	123.7 a	2.7 a	243.8 a
Roots	0.6 a	322.4 a	0.8 a	439.5 a	0.5 a	183.6 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

Table 7. The effect of buthidazole on 'Pioneer 3780' corn height and fresh weight 30 days after applying the herbicide on the root zone vs spraying on the soil surface.[a]

Main effects	Buthidazole (kg/ha)	Zone of application	Plant ht (cm/plant)	Plant fresh wt (g/plant)
1) Zone of application		Root zone	52.00 b	4.25 b
		Soil surface	47.73 a	3.29 a
2) Buthidazole	0		53.01 c	4.52 b
	0.56		50.78 bc	4.03 b
	1.12		47.95 ab	3.22 a
	2.24		47.71 a	3.31 a
Interactions				
	0	Root zone	53.03 c	4.49 cd
	0.56	Root zone	50.5 c	4.05 cd
	1.12	Root zone	50.33 c	3.73 c
	2.24	Root zone	54.15 c	4.72 d
	0	Soil surface	53.00 c	4.55 d
	0.56	Soil surface	51.07 c	4.01 cd
	1.12	Soil surface	45.58 b	2.71 b
	2.24	Soil surface	41.26 a	1.91 a

[a] Means within the same column with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 8. The effect of buthidazole application method on 'Pioneer 3780' corn height and fresh weight after 30 days.[a]

Main effects	Buthidazole (kg/ha)	Method of application	Plant ht (cm/plant)	Plant fresh wt (g/plant)
Method of application		PRE[b]	48.97 b	3.27 b
		PPI[c]	34.56 a	1.62 a
Buthidazole	0		52.00 c	4.32 d
	0.56		50.76 c	3.33 c
	1.12		36.71 b	1.68 b
	2.24		27.59 a	0.44 a
Interactions	0	PRE	52.00 d	4.32 e
	0.56	PRE	58.85 e	4.71 e
	1.12	PRE	50.29 d	3.26 d
	2.24	PRE	34.72 b	0.78 b
	0	PPI	52.00 d	4.32 e
	0.56	PPI	42.67 c	1.94 c
	1.12	PPI	23.13 a	0.12 a
	2.24	PPI	20.46 a	0.10 a

[a] Means within columns with the same letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] PRE = preemergence.

[c] PPI = preplant incorporated.

Table 9. Distribution of total ^{14}C found in various parts of corn plants harvested 3, 8, and 16 days after treatment with ^{14}C-buthidazole at the coleoptile stage.[a]

Plant part	3 days		8 days		16 days	
	(% of total	(dpm/mg)[b]	(% of total)	(dpm/mg)	(% of total)	(dpm/mg)
Methanol - soluble						
First leaf	88.9 c	28864.9 b	61.0 b	13295.2 ab	65.6 b	27461.5 b
Shoot and leaves	3.1 a	673.6 a	18.0 a	313.6 a	19.3 a	123.0 a
Primary roots	2.8 a	78.4 a	2.0 a	98.7 a	1.5 a	65.6 a
Adventitious roots	---	---	10.3 a	668.1 a	2.9 a	122.4 a
Methanol - insoluble						
First leaf	4.9 a	2043.2 a	5.0 a	2123.6 a	6.2 a	1560.1 a
Shoot and leaves	0.1 a	34.9 a	2.2 a	37.4 a	4.0 a	25.1 a
Primary roots	0.2 a	5.7 a	0.2 a	10.3 a	0.2 a	9.8 a
Adventitious roots	---	---	1.3 a	71.0 a	0.3 a	14.9 a

[a] Means within the same plant part and the same parameter with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Calculated as dpm/mg of ^{14}C-buthidazole.

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CHAPTER 3

The Role of Metabolism in Buthidazole Selectivity Between Corn (Zea mays L.) and Redroot Pigweed (Amaranthus retroflexus L.)

ABSTRACT

The metabolism of ^{14}C-buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiaidiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) was studied in corn (Zea mays L.) and redroot pigweed (Amaranthus retroflexus L.) following root application. Corn and redroot pigweed seedlings were placed for 1 day in Hoagland's no. 1 solution containing 10 µCi/L of ^{14}C-buthidazole then transferred into non-labeled nutrient solution and harvested 1, 3, and 6 or 14 days after treatment with buthidazole. In corn, metabolism was also studied following application of 0.2 µCi of ^{14}C-buthidazole to the emerging coleoptile. The first leaf of the emerged treated seedlings was harvested 3, 8, and 16 days after treatment and analyzed for buthidazole and metabolites. After partitioning of methanol-soluble extracts from leaf tiisues against hexane, the bulk of the radioactivity remained in the aqueous phase. Both corn and redroot pigweed metabolized buthidazole in a similar manner but at different rates, yielding as a major metabolite an unknown buthidazole derivative with an Rf value of 0.24 to 0.26 (developing system; chloroform:methanol, 4:1). Corn formed this metabolite very rapidly even in the roots, whereas the buildup of this metabolite in redroot pigweed was very slow, following root application. Minor metabolites with Rf values similar to those of the urea and dihydroxy derivatives of buthidazole were present in both plant species. A metabolite with Rf value corresponding to the amine derivative of buthidazole was detected in corn plants but only after application to the emerging coleoptile. The dihydroxy derivative of buthidazole formed in

redroot pigweed seedlings appeared to be futher metabolized since the radioactivity associated with it decreased as a function of time. A differential rate of buthidazole metabolism in corn and redroot pigweed seems to be very important for the observed selectivity of this herbicide.

INTRODUCTION

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) is a new herbicide with potential for selective weed control in corn, following preemergecne or early postemergence application (1).

A previous study on the pattern of ^{14}C-buthidazole uptake and translocation in corn and redroot pigweed showed that redroot pigweed translocated ^{14}C-buthidazole both acropetally and basipetally whereas corn translocated buthidazole only acropetally into the apoplast (2). Thus, faster herbicide movement from the redroot pigweed roots, the main site of entry, to the leaves, the site of action on photosynthesis (3), was considered to be an important factor contributing to buthidazole selectivity between corn and redroot pigweed (2).

The purpose of this study was to examine the potential contribution of metabolism in the selectivity of buhtidazole between corn and redroot pigweed folowing root application.

MATERIALS AND METHODS

Plant material. Five 'Pioneer 3780' corn and ten redroot pigweed seeds were planted 4.0 and 0.5 cm depp, respectively, into greenhouse soil mixture (1:1:1 soil, sand, peat) in 946-ml waxed cups. After planting, the cups were placed in a greenhouse with temperature ranging from 20 C at night to 33 C during the day without additional artificial light. After emergence, the plants were thinned to 1 plant per cup. Corn seedlings 15 cm tall and

redroot pigweed seedlings 10 cm tall were used for the metabolism studies following root application of ^{14}C-buthidazole. 'Pioneer 3780' corn seeds planted into waxed cups, 1 seed per cup were used for the metabolism study in corn, following application of ^{14}C-buthidazole to the emerging coleoptile.

Application of ^{14}C-buthidazole. Radioactive buthidazole was labeled at the 5 carbon atom of the thiadiazole ring (Figure 1) and had a specific activity of 12.7 mCi/mM. Corn and redroot pigweed seedlings, grown as described, were placed into cups with Hoagland's no. 1 solution (5) containing 10 µCi/L of ^{14}C-buthidazole. The plants remained in the radioactive solution for only 1 day. Then they were transferred and continued to grow in cups containing Hoagland's no. 1 solution without ^{14}C-buthidazole. After treatment, the plants were grown in a growth chamber with a 16-h day and a light intensity of 19 Klux. Day and night temperature was maintained at 25 ± 1 C. Corn plants were harvested 1, 3, and 14 days after treatment and redroot pigweed plants were harvested 1, 3, and 6 days after treatment. ^{14}C-buthidazole metabolism was also studied in corn plants treated with a 5 µl drop containing 0.2 µCi of ^{14}C-buthidazole applied to the emerging coleoptile of corn seedlings as soon as they penetrated the soil surface. The treated corn seedlings continued to grow under the same conditions as those described for the root application study and were harvested 3, 8, and 16 days after the treatemnt with ^{14}C-buthidazole. Since the results of the previous study had revealed that following application of labeled buthidazole to the emerging coleoptile of corn, the bulk of radioactivity was associated with the first leaf (2), only the first leaf of the treated corn seedlings was used for this metabolism study.

Extraction, Separation, and Quantitation of Buthidazole and its Metabolites. Following the ^{14}C-buthidazole treatment the plant tissues were homogenized

in a Sorval-Omni mixer for 5 min in 20 ml of 100% methanol. The homegenates were filtered through Whatman #1 filter paper under vacuum and 15 ml of the filtrate were partitioned against 15 ml of hexane. After partitioning, the bulk of radioactivity (more than 95%) remained in the aqueous phase. The roots of harvested corn and redroot pigweed seedlings were not used for the metabolism studies because the amount of radioactivity associated with them was very low, as reported earlier (2). Only the roots of corn plants harvested 1 day after root application were examined for metabolism since in the aforementioned study the amount of radioactivity associated with them was relatively high, accounting for 40% of the total radioactivity detected (2). The procedure for extracting buthidazole and metabolites form these roots, was similar to the one used for the leaves and stems of the treated plants. One hundred µl samples from the aqueous phase of each extract were spotted on Thin Layer Chromatography (TLC) paltes precoated with a silica gel G layer 250 µm thick. The plates were developed in a solvent system containing 80% chloroform and 20% methanol (4:1) and localization of the separated metabolites was determined by both radioautography and TLC scanning by using a Berthold LB scanner with a slit width of 2 mm. Quantitation of the radioactivity present in each metabolite was done by integration of the area under the peak obtained for each spot, using the TLC scanner. The results of quantitation were expressed as percent of the total radioactivity found in the 100 µl samples of each extract.

Identification of Metabolites. The TLC absorbant containing the ^{14}C labeled methanol-extractable spot was removed from the TLC plate and extracted with 2 ml of methanol, centrifuged to 500 g, and filtered through glass fiber filter under vacuum. The filtrates were evaporated down to 100 µl under nitrogen. Then the separated metabolites were co-chromatographed

with known compounds on 20 x 20 cm plates (Redi/Plate, Analtech, Inc.) coated with silica gel GF (250 μm) in the same solvent system of chloroform to methanol in a 4:1 ratio. Localization of the metabolites was achieved by exposure to ultraviolet light (UV 254 nm). Presence of small amounts of unknown ^{14}C-metabolites was confirmed by TLC scanning. The following known buthidazole derivatives used for identification were kindly provided by Velsicol Chemical Corporation: a) Analytical grade buthidazole 98.7% pure by infrared spectroscopy (IR). b) Buthidazole DiOH (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4,5-dihydroxy-2-imidazolidinone, 100% pure by TLC. c) Buthidazole methylurea (3-[5-(1,1-dimehtylethyl)-1,3,4-thiadiazol-2-yl]-1-methyl)urea, 99% pure by TLC. d) Buthidazole urea (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-urea, 99% pure by TLC. and e) Buthidazole amine [5-amino-2-(1,1-dimethylethyl)-1,3,4-thiadiazole], 99.8% pure by Liquid chromatography.

RESULTS AND DISCUSSION

The Rf values of the analytical reference standards for identification of the unknown metabolites are shown in Table 1. The methanol-soluble metabolites detected in corn plants, 1, 3, and 14 days after root application are shown in Table 2. Corn metabolized buthidazole very fast, even in the roots, yielding a major metabolite with Rf value of 0.26 and two minor metabolites with Rf values of 0.57 and 0.62, beside the unmetabolized ^{14}C-buthidazole. From the data presented in Table 2 and in Figures 2 and 3, it is clear that as the amount of radioactivity in unknown #1 increases, the radioactivity in the parent buthidazole decreases. Fourteen days after the treatment 53% of the total radioactivity was present in the major metabolite #1, whereas the level of the unmetabolized buthidazole had dropped to 27% of the total radioactivity (Table 2). A two-fold increase of the radioactivity in unknown #3 was also noticeable 3 days after

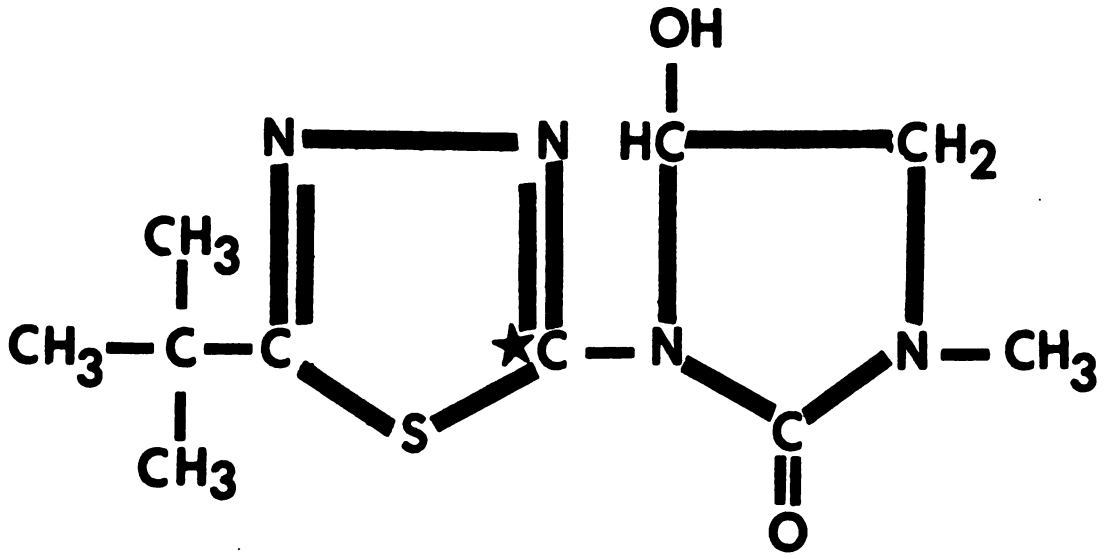
treatment and then remained constant to 14 days (Table 2). Following application of ^{14}C-buthidazole to the emerging coleoptile, the number of detected metabolites of buthidazole in corn appeared to be higher, with two new metabolites present (Table 3). The metabolite with Rf value of 0.25 appeared again to be the major metabolite, accounting for 52% of the total radioactivity 3 days after application of ^{14}C-buthidazole (Table 3). However, the amount of radioactivity associated with the unmetabolized buthidazole and the five metabolites remained more or less constant with time. This may be related to the apoplastic translocation paterrn of buthidazole in corn. The two new metabolites had Rf values of 0.46 and 0.53, respectively. Comparison of the Rf values of the standard reference compounds to those of the buthidazole metabolites detected in corn (Tables 1,2,3) indicates that unknown #3 (Table 3) may be the amine derivative of btuhidazole whereas unknowns #2 and 3 (Table 2) or unknowns #4 and 5 (Table 3) may be the urea and dihydroxy derivatives of buthidazole. Urea and dihydroxy derivatives of buthidazole have been reported as minor metabolites of buthidazole in metabolism studies with sugarcane (4). Data presented in Table 4 indicates that metabolites with Rf values similar to those of the urea and dihydroxy buthidazole derivatives were also present in redroot pigweed. However, the amount of radioactivity associated with the dihydroxy derivative of buthidazole decreases with time indicating that this metabolite might be further metabolized in redroot pigweed (Table 4). The metabolite with Rf value 0.25 appears to be the major buthidazole derivative but its formation is very slow as compared to its formation in corn (Tables 2,3,4 and Figures 2 and 3). Thus, 1 day after root application unknown #1 is present only as 4% of the total radioactivity, but this increased to 32.6% of the total at 6 days (Table 4). The unknown #2

with an Rf value of 0.47 (Table 4) appeared to be similar to unknown #2 formed in corn (Table 3).

The major metabolite of buthidazole in corn and redroot pigweed with Rf values ranging from 0.24 to 0.26 (Tables 2,3,4) did not appear to have chromatographic properties similar to any of the buthidazole derivatives shown in Table 1. Attempts to identify this metabolite have been unsuccessful so far. The desmethyl derivative of buthidazole has been reported as the major metabolite of buthidazole in sugarcane (4). N-demethylation of other thiadiazolyl herbicides has also reported and it is considered very important for the selective action of these herbicides in grass species (6,7). Whether the unknown metabolite with Rf value of 0.24 to 0.26 corresponds to the N-demethylated derivative or to an other unknown metabolite of buthidazole is not known at present.

In summary we concluded that corn and redroot pigweed metabolized buthidazole similarly but at different rates. An unknown metabolite with an Rf value of 0.26 to 0.26 appeared to be the major metabolite of buthidazole in corn following both root and coleoptile applications. Formation of this metabolite in corn was very fast, occuring even in the roots, 1 day after root application of ^{14}C-buthidazole. The buildup of this metabolite in redroot pigweed was slow apparently contributing to the observed selectivity of buthidazole between these two species. Minor metabolites with Rf values similar to those of the urea and dihydroxy derivatives of buthidazole were present in both corn and redroot pigweed. A minor metabolite in corn following application to the emerging coleoptile, appeared to be the amine derivative of buthidazole. A differential rate of buthidazole metabolism in corn and redroot pigweed, combined with the differential rate of translocation, reported earlier, seem to be two very important factors contibuting to buthidazole selectivity between these two species.

Figure 1. Chemical structure and chemical name of the herbicide buthidazole. The asterisk(*) indicates the radioactive labeled carbon atom (^{14}C).



3-[5-(1,1-dimethylethyl)-1,3,4- thiadiazol-2-yl]-4- hydroxy-
1-methyl-2- imidazolidinone

BUTHIDAZOLE

Figure 2. Radioscans of thin-layer chromatograms of extracts of redroot pigweed and corn treated with ^{14}C-buthidazole, 1 day after application to the roots. The developing system was Chloroform : Methanol (4:1).

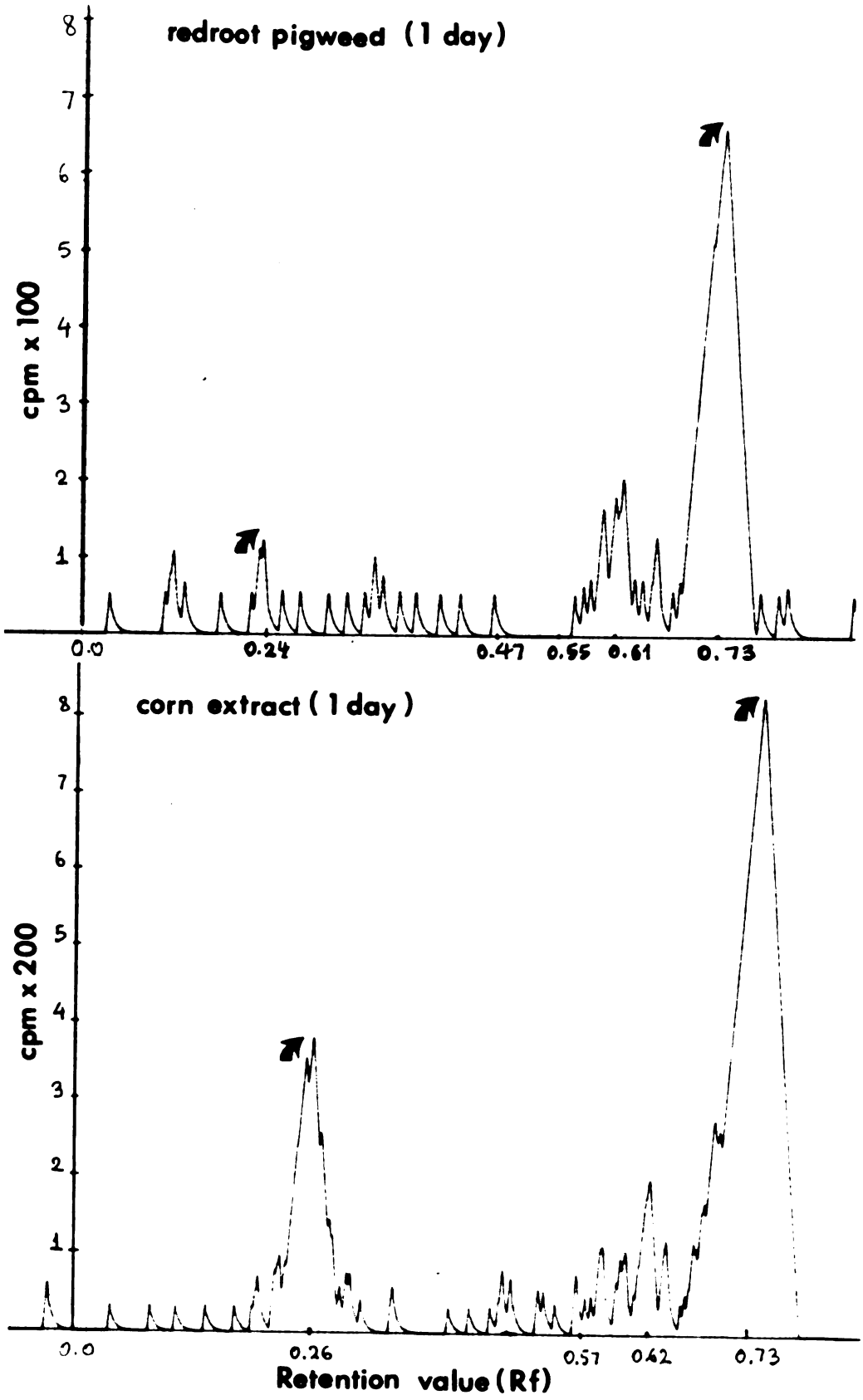
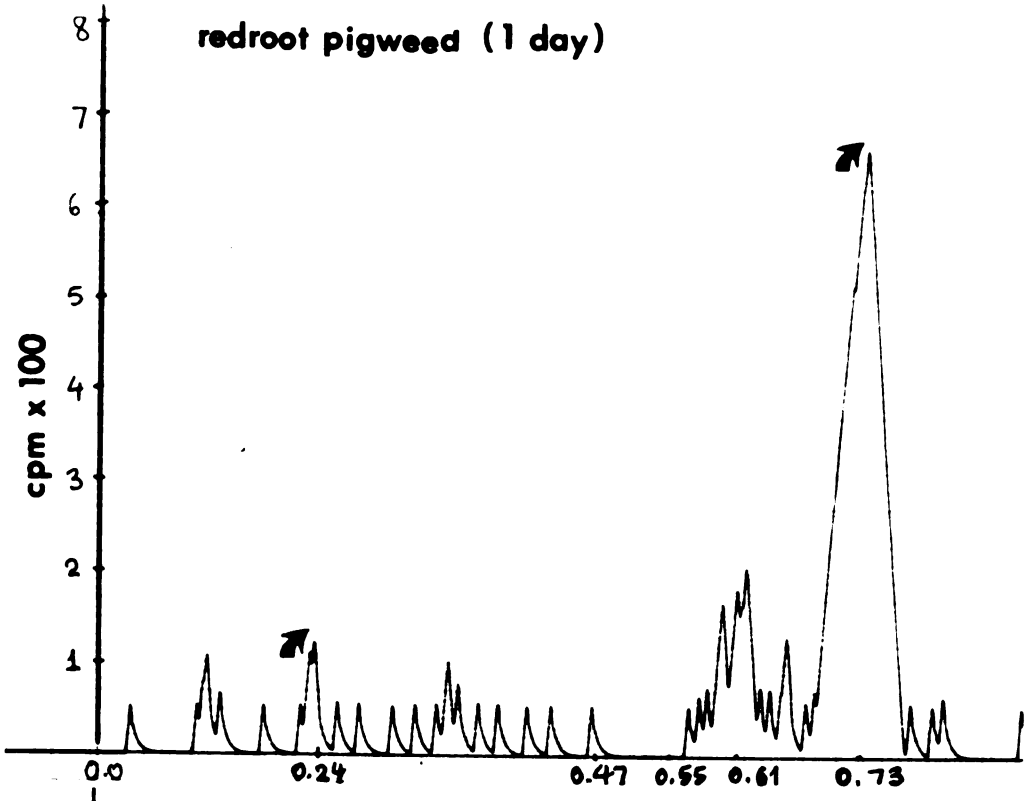

redroot pigweed (1 day)
cpm x 100
8
7
6
5
4
3
2
1
0.0
0.24
0.47
0.55
0.61
0.73
corn extract (1 day)
cpm x 200
8
7
6
5
4
3
2
1
0.0
0.26
0.57
0.62
0.73
Retention value (Rf)

Figure 2. Radioscans of thin-layer chromatograms of extracts of redroot pigweed and corn treated with ^{14}C-buthidazole, 1 day after application to the roots. The developing system was Chloroform : Methanol (4:1).


redroot pigweed (1 day)
cpm x 100
8
7
6
5
4
3
2
1
0.0
0.24
0.47
0.55
0.61
0.73

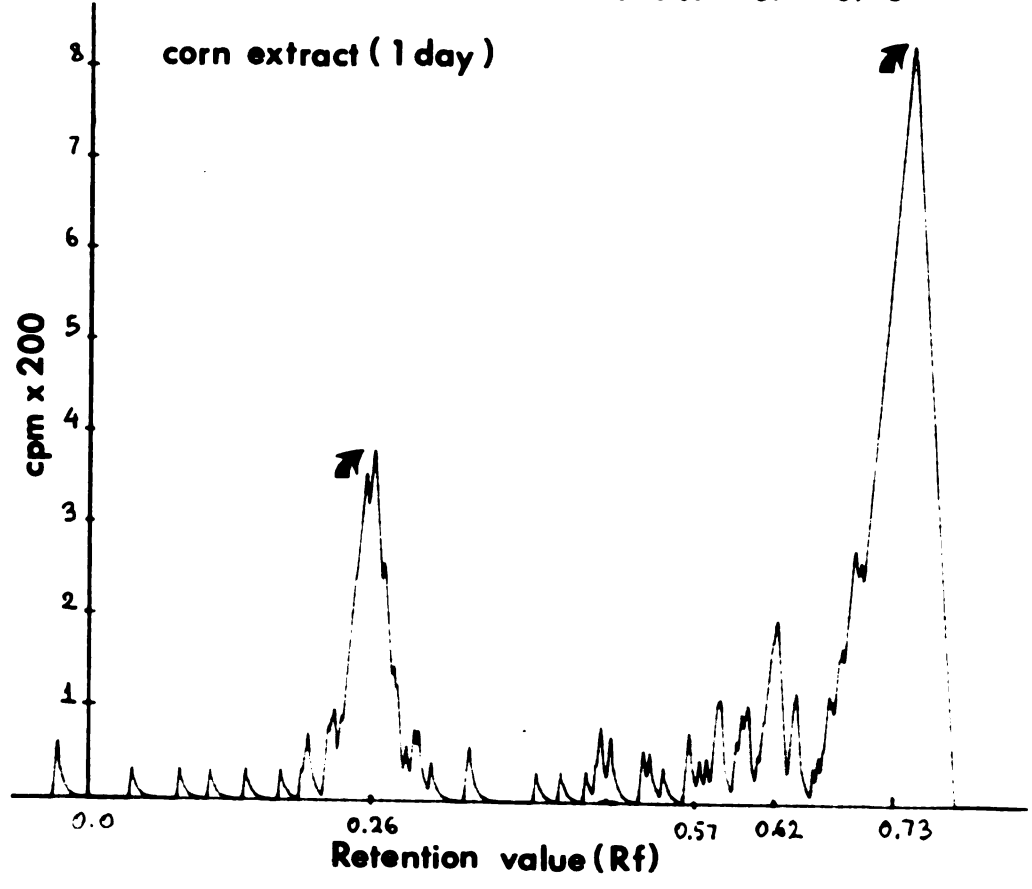

corn extract (1 day)
cpm x 200
8
7
6
5
4
3
2
1
0.0
0.26
0.57
0.62
0.73
Retention value (Rf)

Figure 3. Radioscans of thin-layer chromatograms of extracts of redroot pigweed and corn treated with ^{14}C-buthidazole, 3 days after application to the roots. The developing system was Chloroform : Methanol (4:1).

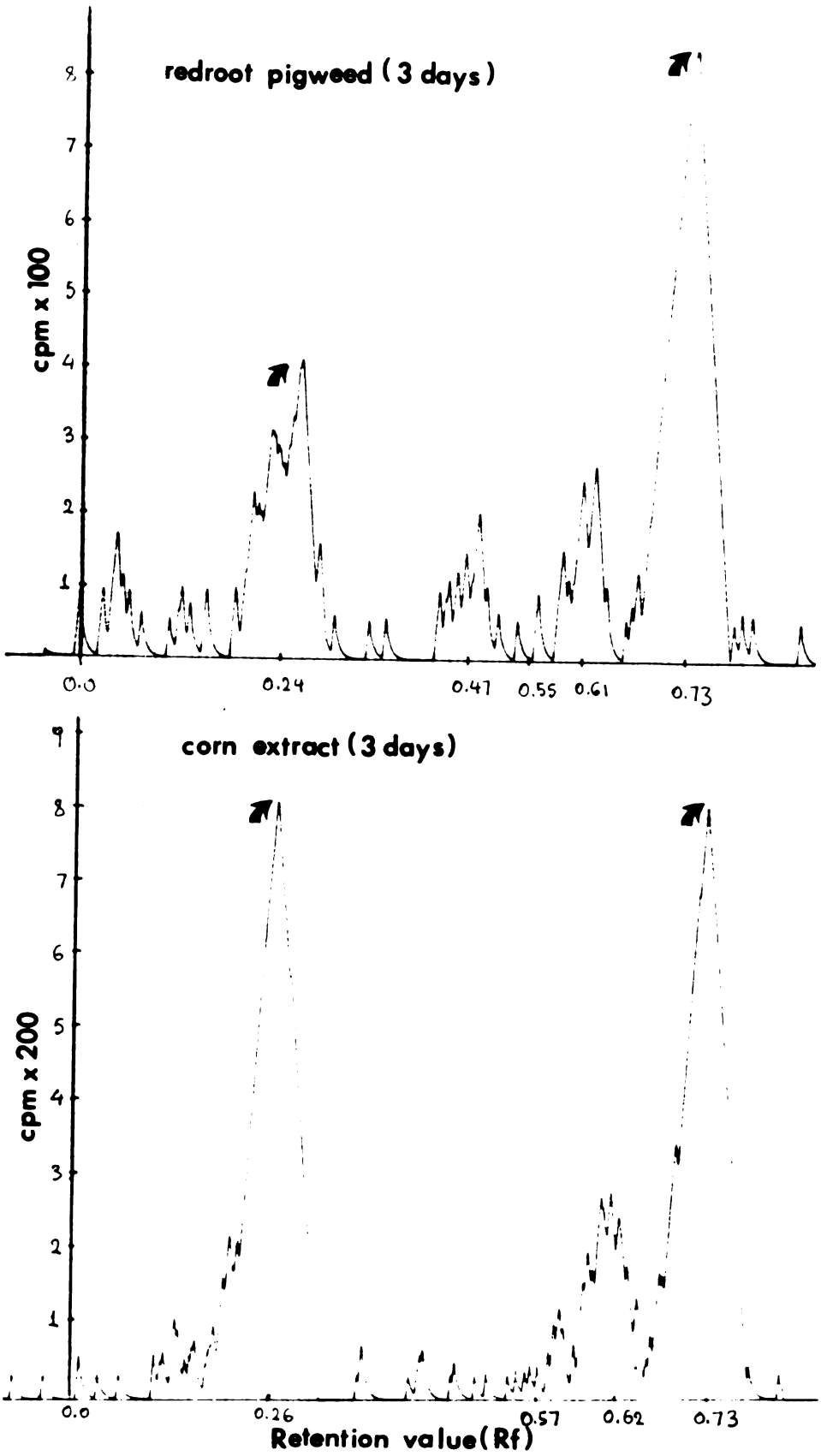

redroot pigweed (3 days)
cpm x 100
0.0
0.24
0.47
0.55
0.61
0.73
corn extract (3 days)
cpm x 200
0.0
0.26
0.57
0.62
0.73
Retention value(Rf)

Table 1. Rf values of analytical reference standards used for identification of unknown buthidazole metabolites.

Analytical Standard	Rf value*
Buthidazole amine	0.54
Buthidazole urea	0.56
Buthidazole DiOH	0.62
Buthidazole Methylurea	0.65
Buthidazole	0.71 to 0.73

* Developing system; Chloroform: Methanol (4:1).

Table 2. Methanol-soluble metabolites of ^{14}C-buthidazole in corn plants 1, 3, and 14 days after root application[a].

		Days after treatment			
		1		3	14
Metabolite	Rf value*	Leaves	Roots	Leaves	Leaves
		(% of total radioactivity)			
Unknown #1	0.26	30.4 e	28.2 e	43.5 f	53.0 g
Unknown #2	0.57	2.4 ab	2.5 ab	1.2 a	5.7 bc
Unknown #3	0.62	6.9 c	3.3 b	13.3 d	13.9 d
Buthidazole	0.73	60.3 h	66.0 h	42.0 f	27.4 e

[a] Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. Methanol-soluble metabolites of ^{14}C-buthidazole in the first leaf of corn plants treated with buthidazole applied to the emerging coleoptile 3, 8, and 16 days after treatment[a].

Metabolite	Rf value*	Days after treatment 3	8	16
		(% of total radioactivity)		
Unknown #1	0.25	52.0 h	38.4 d	44.6 ef
Unknown #2	0.46	0.8 a	1.4 a	0.8 a
Unknown #3	0.53	0.4 a	1.9 a	0.4 a
Unknown #4	0.57	0.4 a	1.4 a	0.4 a
Unknown #5	0.63	3.6 ab	9.4 c	5.2 b
Buthidazole	0.74	42.8 e	47.5 fg	48.6 g

[a] Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

* Developing system ; Chloroform : Methanol (4:1).

Table 4. Methanol-soluble metabolites of ^{14}C-buthidazole in redroot pigweed leaves 1, 3, and 6 days after root application[a].

Metabolite	Rf value*	Days after treatment 1	3	6
		(% of total radioactivity)		
Unknown #1	0.24	4.0 a	22.4 de	32.6 f
Unknown #2	0.47	3.7 a	6.5 abc	11.2 c
Unknown #3	0.55	1.8 a	2.4 a	5.0 ab
Unknown #4	0.61	24.7 e	18.3 d	10.6 bc
Buthidazole	0.73	65.8 i	50.4 h	40.6 g

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CHAPTER 4

Localizing the Metabolic Site of Action of Two Thiadiazolyl Herbicidal Derivatives

ABSTRACT

Enzymatically isolated leaf cells from navy beans (Phaseolus vulgaris L., cv. 'Tuscola') were used to study the effect of buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) and tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethyl-urea) on photosynthesis, ribonucleic acid (RNA), protein, and lipid synthesis. The incorporation of $NaH^{14}CO_3$, ^{14}C-uracil, ^{14}C-leucine, and ^{14}C-acetic acid as substrates for the respective metabolic process were measured. Time-course and concentration studies included incubation periods of 30, 60, and 120 minutes and concentrations of 0.1, 1, 10, and 100 µM of both herbicides. Photosynthesis was very sensitive to both buthidazole and tebuthiuron and was inhibited in 30 min by 0.1 µM of both herbicides. RNA and lipid syntheses were inhibited 50 and 87%, respectively, by buthidazole and 42 and 64%, respectively, by tebuthiuron after 120 min at 100 µM concentration. Protein synthesis was not affected by any herbicide at any concentration or any exposure time period. The inhibitory effects of buthidazole and tebuthiuron on RNA and lipid syntheses may be involved in the ultimate herbicidal action of these herbicidal chemicals.

INTRODUCTION

Following the original description of a procedure for the preparation of physiologically active tobacco mesophyll cells by Takebe et al.(1), Jensen and coworkers (2,3) developed techniques for the isolation of mesophyll cells that could photosynthesize or absorb and incorporate protein and ribonucleic acid precursors. Since suspensions of separated leaf cells can be handled like bacteria or unicellular algae, they offer several advantages over whole plants in studying the mode of action of herbicides. Thus, use of single cells in

studies on the mode of action of several herbicides has already been reported (4,5,6,7).

Compounds containing 1,3,4-thiadiazolyl group in their molecular structures have been reported to be phytotoxic (8,9). Original work in Japan revealed that 1,1-dimethyl-3-(5-tert-butyl-1,3,4-thiadiazol-2-yl)urea showed the strongest herbicidal activity among the thiadiazolyl urea derivatives tested (8). Later on, tebuthiuron (N-[5-(1,1-dimethyl-ethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea) and buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) were synthesized and developed as industrial herbicides in the United States (10,11). These two herbicides have also shown promise for agricultural uses. Thus, buthidazole has shown potential for selective weed control in corn (Zea mays L.) following preemergence or early post-emergence application at low rates and in dormant alfalfa (Medicago sativa L.) following postemergence application (11). Tebuthiuron has been reported promising for broad spectrum weed control in sugarcane (Saccharum officinarum L.) following preemergence application (12).
The chemical structures of buthidazole and tebuthiuron are shown in Figure 1.

The purpose of this research was to determine the primary metabolic site of the herbicidal action of buthidazole and tebuthiuron by examining the effects of these herbicides on photosynthesis, RNA, protein, and lipid syntheses of isolated navy bean cells under various time-course and concentration conditions.

MATERIALS AND METHODS

Plant material. Navy bean (Phaseolus vulgaris L., cv. 'Tuscola') seeds were grown in a mixture of vermiculite and greenhouse soil (1:1) in waxed cups at a temperature 23 ± 1 C. One day after emergence the cups were transferred to a growth chamber with 25 ± 1 C temperature and light intensity of 16 Klux

at the level of the primary leaves. Light was supplied by a combination of fluorescent and incandescent lamps for a 16-hr period which was followed by an 8-hr dark period.

Media. The maceration, wash, and incubation media were the ones used by Ashton et al.(6), modified in some cases as suggested by Porter and Bartels (5). The maceration medium for all cell preparations contained 2% macerase (Calbiochem) with 0.3% potassium dextran sulfate (Calbiochem) and 0.7 M sorbitol at pH 5.8. The maceration medium was made up daily prior to use from a 0.7 M stock sorbitol solution, adding the appropriate amounts of the maceroenzyme and dextran sulfate. The wash medium contained 0.65 M sorbitol, 1 mM KNO_3, 0.2 mM KH_2PO_4, 0.1 mM $MgSO_4$, 1 mM $CaCl_2$, 1 μM KI, and 1 μM $CuSO_4$, adjusted to pH 5.8. The incubation medium was identical to wash medium but contained 0.625 M instead of 0.65 M sorbitol.

The incubation medium was buffered with 0.05 M HEPES adjusted to pH 7.2 with 0.1 M KOH for the photosynthesis studies. For protein, RNA, and lipid synthesis studies, the incubation medium was buffered with 50 mM MES and adjusted to pH 5.8 with KOH. The wash and incubation stock media were made up weekly.

Isolation of cells. Cells were isolated according to the method of Jensen et al.(2) as modified by Ashton et al.(6) and by Porter and Bartels (5). Primary leaves from 7- to 10-day-old bean plants wer harvested 4 to 5 hr after initiation of the light period, rinsed in distilled water, blotted, deveined, and cut into 2 x 3 mm pieces. Five grams of tissue were then vacuum infiltrated with 30 ml of maceration medium untill they were fully infiltrated. After vacuum infiltration the leaf tissue was filtered through a 242 μm nylon net, transferred to another 30 ml of maceration medium, and slowly stirred on a magnetic stirrer for 10 min. The solution was again filtered through the same nylon net and the filtrate

was discarded. The leaf tissue was transferred again to 30 ml of maceration medium and it was stirred for 50 min. The cells released during this period were filtered again through the same net and the tissue was washed with 20 ml of maceration medium. The released cells were centrifuged for 3 min at 60 x g at room temperature. The supernatant fraction was removed by suction and the cells were washed three times with 10 ml aliquots of wash medium by centrifugation at 60 x g for 3 min at room temperature. The supernatant solution was removed by suction and the cells were made up to a desired volume with incubation medium so that the cell preparations used for the assays contained 0.04 to 0.06 mg of chlorophyll per ml, or 0.08 to 0.1 mg of chlorophyll per assay. In each assay the assaying mixture contained 2 ml of the cell preparation in a 25 ml Erlenmyer flask, 0.1 ml of the radioactive substrate and 0.05 ml of the herbicide solution, making a volume of 2.15 ml. For the chlorophyll determination 1.0 ml of the cell suspension was added to 4 ml of 80% acetone and centrifuged. Then the chlorophyll content was determined by the method described by Arnon (13).

Metabolic studies

Photosynthesis. Photosynthesis was assayed according to the method of Jensen et al. (2) as modified by Ashton et al.(6). The cells were incubated with 5 μCi $NaH^{14}CO_3$ (sp. act. 10 mCi/mmole) containing 6.0 μM of $NaH^{12}CO_3$. The erlenmyer flasks with the assay mixtures were sealed and placed in a shaking waterbath at 25 C. The flasks were illuminated from above with a combination of fluorescent and incandescent lamps with an intensity of 4.5 Klux at the level of the flasks. After the specific incubation periods used, a 100 μl sample was removed with a pipet and placed on a 2.3 cm Whatmann 3 μM filter paper disc. The discs were dried under a hair dryer, acidified with 100 μl of 88% formic acid and dried again for 1 hr. Radioactivity was determined by radioassaying the discs by liquid scintillation

spectrometry. Photosynthesis was calculated as cpm of $^{14}CO_2$ fixed per mg of chlorophyll.

Protein and RNA synthesis. Incorporation of ^{14}C-leucine and ^{14}C-uracil was determined by the method of Francki et al. (3) as modified by Ashton et al. (6). One µCi of L-[U-^{14}C]-leucine (sp. act. 70 mCi/mmole) and 5 µCi of [2-^{14}C]-uracil (sp. act. 65 mCi/mmole) were added to the cells. Incubation conditions were the same as described for photosynthesis. Five hundred µl samples were collected and added to 1.9 ml of ice-cold 12% trichloroacetic acid (TCA) containing 50 mM L-leucine for the protein synthesis and 30 mM uracil for the RNA synthesis study and left overnight at 4 C. The protein and ribonucleic acid precipitates were then collected by filtering through 2.1 cm glass fiber filter discs (Arthur Thomas), washed three times with ice-cold 10% TCA, three times with 80% ethanol, once with acetone, and twice with diethyl ether. The discs were then put in vials, dried in an oven for 30 min and the radioactivity determined by liquid scintillation spectrometry. Protein and RNA syntheses were calculated as cpm per mg of chlorophyll.

Lipid synthesis. Lipid synthesis was determined by the method of Ashton et al. (6). One µCi of [1,2-^{14}C]acetic acid, sodium salt (sp. act. 56.2 mCi/mmole) was added to the cells. Incubation conditions were the same as in photosynthesis. Five hundred-µl samples were collected in 2 ml of 0.35 M H_2SO_4 and 0.05 M CH_3COOH in conical centrifuge tubes. The samples were allowed to sit in the acid for at least 15 min and they were centrifuged for 10 min at 160 x g at room temperature. The supernatant fraction was removed by suction and 4 ml of $CHCl_3/CH_3OH$ (2:1) was added and mixed. The tubes were stoppered and left overnight at room temperature. Two ml of distilled water was added and the mixture was centrifuged for 5 min at 160 x g at room temperature. The top layer was removed by suction.

This procedure was repeated three times. The chloroform solution was filtered through glass fiber filter discs into vials and the discs were washed two times with $CHCl_3/CH_3OH$ (2:1). The filtered solution was dried under a current of air, and the radioactivity in the lipid fraction was determined by liquid scintillation spectrometry. Lipid synthesis was calculated as cpm per mg of chlorophyll.

Radioactivity determination. Radioactivity was determined by adding 10 ml of Aqueous Counting Scintillant (ACS, Amersham) to samples for radioassay with a Beckman LS 8100 Liquid Scintillation Spectrometer.

Time-course and concentration studies with buthidazole and tebuthiuron. Analytical grade (more than 99% pure) buthidazole and tebuthiuron were diluted in 5 ml of ethanol and made up to volume with distilled water. Herbicide concentrations of 0.1, 1, 10, and 100 μM were used in all assays. The assay mixtures in all studies were incubated for time periods of 30, 60, and 120 minutes. All experiments were repeated three times. Data presented are the means of these three experiments analyzed for analysis of variance in a two-way factorial design. Duncan's multiple range test was used to separate the means.

RESULTS AND DISCUSSION

The effects of buthidazole and tebuthiuron on four metabolic processes of isolated bean cells were examined (Tables 1 through 4). Inhibition of photosynthesis was very rapid, reaching maximum levels in 30 min incubation time with the high concentrations of both herbicides. However, in the case of the low concentration 0.1 μM, the inhibition rate increased from 27 and 30% after 30 min to 44 and 46% after 60 min of incubation with buthidazole and tebuthiuron, respectively (Table 1). Although the results clearly suggest that both herbicides are strong photosynthetic inhibitors at high concentrations, the inhibition rates obtained are

somewhat lower than those reported by Ashton et al.(6) for other well-known photosynthetic inhibitors such as atrazine, bromacil, and monuron used at the same concentrations. However, buthidazole and tebuthiuron caused greater inhibition of photosynthesis at the low concentrations of 0.1 μM compared to the aforementioned herbicides in the study by Ashton et al. (6). Differences in absorption or subcellular transport, although not documented, may account for the observations. In another study, both buthidazole and tebuthiuron were found comparable to atrazine and diuron as inhibitors of photosynthetic electron transport in isolated spinach chloroplasts (14).

Significant inhibition of RNA synthesis was found to be caused by buthidazole at 1, 10, and 100 μM and tebuthiuron at 10 and 100 μM (Table 2). Inhibition of RNA by buthidazole and tebuthiuron did not appear to be a function of the incubation time period since the inhibition percentages remained unchanged for all incubation times (Table 2).

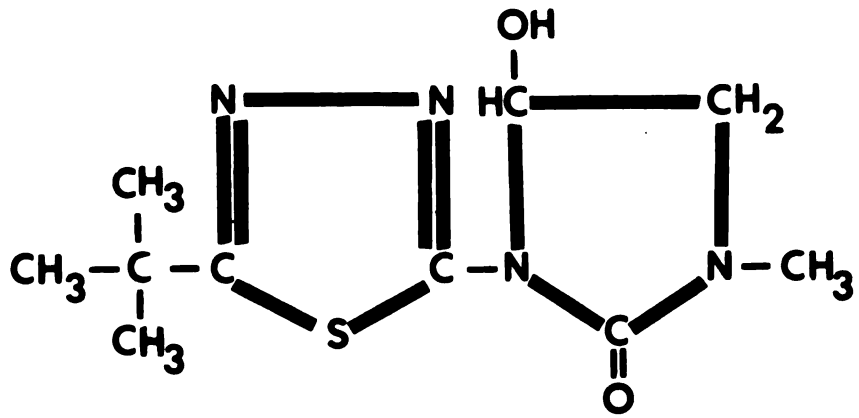
Protein synthesis was not affected by any herbicide even at the maximum concentration and maximum exposure time (Table 3). This appears to be an exception to the behavior of photosynthetic inhibitors used as herbicides as reported by Ashton et al. (6) where they found significant inhibitions of protein synthesis by atrazine, bromacil, and monuron at high concentrations.

Lipid synthesis was inhibited significantly by the high concentrations (10 and 100 μM) of both herbicides reaching levels of 84 and 64% inhibition for buthidazole and tebuthiuron at 100 μM, respectively (Table 4). A slight stimulation of lipid synthesis by the lower concentration of 0.1 μM, which inhibited photosynthesis, was found with tebuthiuron at any incubation time and with buthidazole at the maximum exposure time. This agrees with the results of Ashton et al. (6) for other herbicidal photosynthetic

inhibitors.

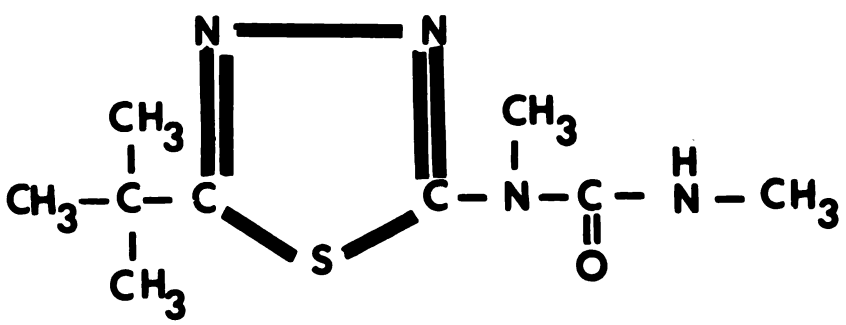
The lowest concentration of buthidazole and tebuthiuron that inhibited any of the four metabolic processes studied was 0.1 μM which at 120 min inhibited photosynthesis 43 and 35% respectively (Table 1). Protein and RNA syntheses are essentially unaffected by this concentration at any exposure time (Tables 2 and 3). At the highest concentration of 100 μM and maximum exposure time of 120 min, photosynthesis was inhibited 87 and 81%, RNA synthesis 50 and 42%, protein synthesis 11 and 10%, and lipid synthesis 84 and 64% by buthidazole and tebuthiuron, respectively.

The results of this study indicate that both buthidazole and tebuthiuron act in a similar manner. Photosynthesis was the most sensitive and first metabolic process inhibited. The inhibitory effects on RNA and lipid syntheses caused by both herbicides at high concentrations may be involved in the ultimate herbicidal action of buthidazole and tebuthiuron. Protein synthesis was not affected by any of these herbicides.



3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone

buthidazole



N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea

tebuthiuron

Figure 1. Chemical structures of the herbicides buthidazole and tebuthiuron

Table 1. The effect of buthidazole and tebuthiuron on photosynthesis of isolated bean cells[a].

Incubation Time	Buthidazole	$^{14}CO_2$ Fixation	Inhibition	Tebuthiuron	$^{14}CO_2$ Fixation	Inhibition
(min)	(μM)	(cpm/mg Chl)	(%)	(μM)	(cpm/mg Chl)	(%)
	0	9514 e	0	0	14077 ef	0
	0.1	6944 d	27	0.1	9827 cd	30
30	1	2667 ab	72	1	8019 cd	43
	10	1528 a	84	10	3904 ab	72
	100	1333 a	86	100	2231 a	84
	0	24417 h	0	0	29288 i	0
	0.1	13653 f	44	0.1	15981 f	46
60	1	5750 cd	77	1	13288 ef	55
	10	2694 ab	89	10	11346 de	61
	100	2958 ab	88	100	6654 bc	77
	0	27319 i	0	0	36711 j	0
	0.1	15625 g	43	0.1	24019 h	35
120	1	7431 d	73	1	20500 g	44
	10	4000 bc	86	10	9288 cd	75
	100	3792 bc	87	100	7058 bc	81

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. The effect of buthidazole and tebuthiuron on RNA synthesis of isolated bean cells[a].

Incubation Time	Buthidazole	^{14}C-uracil Incorporated	Inhibition	Tebuthiuron	^{14}C-uracil Incorporated	Inhibition
(min)	(μM)	(cpm/mg Chl)	(%)	(μM)	(cpm/mg Chl)	(%)
	0	23348 gh	0	0	11196 cd	0
	0.1	21015 efg	10	0.1	10609 bc	5
30	1	16606 bcd	29	1	9609 bc	14
	10	14379 ab	38	10	8380 ab	25
	100	12894 a	45	100	6837 a	39
	0	25727 hi	0	0	17359 f	0
	0.1	22803 fgh	11	0.1	15174 ef	13
60	1	18651 cde	28	1	15728 ef	9
	10	16197 abcd	37	10	12152 cd	30
	100	15636 abc	39	100	10935 bcd	37
	0	30000 j	0	0	23228 g	0
	0.1	28500 ij	5	0.1	24022 g	0
120	1	24000 gh	20	1	23576 g	0
	10	19500 def	35	10	16837 f	28
	100	15000 ab	50	100	13522 de	42

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. The effect of buthidazole and tebuthiuron on protein synthesis of isolated bean cells[a].

Incubation Time	Buthidazole	^{14}C-leucine Incorporated	Inhibition	Tebuthiuron	^{14}C-leucine Incorporated	Inhibition
(min)	(μM)	(cpm/mg Chl)	(%)	(μM)	(cpm/mg Chl)	(%)
	0	12579 a	0	0	17667 a	0
	0.1	12105 a	4	0.1	18361 a	0
30	1	11842 a	6	1	17339 a	2
	10	11614 a	8	10	16028 a	9
	100	10842 a	14	100	15194 a	10
	0	16474 b	0	0	24028 ab	0
	0.1	16281 b	1	0.1	24528 ab	0
60	1	15824 b	4	1	24972 ab	0
	10	15526 b	6	10	24278 ab	0
	100	15192 b	8	100	24000 ab	1
	0	28737 d	0	0	35361 c	0
	0.1	27123 cd	6	0.1	32167 bc	9
120	1	26719 cd	7	1	33167 bc	6
	10	26403 c	8	10	33805 bc	4
	100	25702 c	11	100	31750 bc	10

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 4. The effect of buthidazole and tebuthiuron on lipid synthesis of isolated bean cells[a].

Incubation Time	Buthidazole	^{14}C-acetate Incorporated	Inhibition	Tebuthiuron	^{14}C-acetate Incorporated	Inhibition
(min)	(μM)	(cpm/mg Chl)	(%)	(μM)	(cpm/mg Chl)	(%)
	0	5286 bc	0	0	6026 ab	0
	0.1	4939 bc	7	0.1	6897 abc	0
30	1	4224 abc	20	1	5487 ab	9
	10	3326 abc	37	10	4461 ab	26
	100	2326 a	56	100	3538 a	41
	0	5918 cd	0	0	8154 abc	0
	0.1	5796 cd	2	0.1	11205 bcd	0
60	1	4020 abc	32	1	8026 abc	2
	10	2306 a	61	10	6282 ab	23
	100	1755 a	70	100	4154 ab	49
	0	11102 e	0	0	15641 d	0
	0.1	11714 e	0	0.1	17154 d	0
120	1	7816 d	30	1	13487 cd	14
	10	2918 ab	74	10	8333 abc	47
	100	1755 a	84	100	5564 ab	64

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

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CHAPTER 5

Inhibition of Photosynthetic Electron Transport in Isolated Spinach Chloroplasts by Two 1,3,4-Thiadiazolyl Derivatives

ABSTRACT

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) and tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea), are two new promising herbicides for selective weed control in corn (Zea mays L.) and sugarcane (Saccharum officinarum L.), respectively. The effects of these two compounds on various photochemical reactions of isolated spinach (Spinacea oleracea L.) chloroplasts were studied at concentrations of 0, 0.05, 0.5, 5, and 500 μM. Buthidazole and tebuthiuron at concentrations higher than 0.5 μM inhibited uncoupled electron transport from water to ferricyanide or to methylviologen very strongly. Photosystem II-mediated transfer of electrons from water to oxidized diaminodurene, with 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) blocking photosystem I, was inhibited 34 and 37% by buthidazole and tebuthiuron, respectively, at 0.05 μM. Inhibition of photosystem I-mediated transfer of electrons from diaminodurene to methylviologen, with 3,4-dichlorophenyl-1,1-dimethylurea (DCMU) blocking photosystem II, was insignificant with both herbicides at any concentration tested. This suggests that both buthidazole and tebuthiuron do not inhibit electron transport through photosystem I. Transfer of electrons from catechol to methylviologen in hydroxylamine-washed chloroplasts was inhibited 50 and 47% by buthidazole and tebuthiuron, respectively, at 0.5 μM. The data indicate that the inhibition of electron transport by both herbicides is primarily at the reducing side of photosystem II. However, since catechol is an electron donor at the oxidizing side of photosystem II, between water and chlorophyll a_{680}, and lower inhibition levels were

observed in the last study (catechol to methylviologen), it may be that there is also a small inhibition of the mechanism of water oxidation by both herbicides.

INTRODUCTION

Substituted 1,2,4- and 1,3,4-thiadiazoles have been reported to possess herbicidal activity (5,10). In the case of 1,3,4-thiadiazoles, this activity was found to be strongly associated with the 5-(1,1-dimethylethyl)-1,3,4-thiadiazole nucleus of the molecule (10). At present two 5-(1,1-dimethylethyl)-1,3,4-thiadiazolyl derivatives are marketed commercially as herbicides for industrial weed control under the common names buthidazole and tebuthiuron (Figure 1). These two herbicides have also shown promise for agricultural uses. Thus buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) has shown promise for selective weed control following preemergence application in corn (Zea mays L.) and postemergence application in established alfalfa (Medicago sativa L.) (1). Tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea) has exhibited potential for rangeland brush control (4) and for broad spectrum weed control in sugarcane (Saccharum officinarum L.) (14).

Inhibition of photosynthesis appears to be involved in the action of 1,3,4-thiadiazolyl herbicides. Thus buthidazole inhibited corn photosynthesis in vivo following either pre- or post-emergence application (8). Prevention of starch accumulation in bundle sheath chloroplasts and some ultrastructural disruption of mesophyll chloroplasts of corn plants treated with buthidazole applied postemergence were also observed in the previous study (8). Phytotoxicity symptoms suggested that inhibition of photosynthesis is also the mode of action of tebuthiuron (2).

Interference with photo-induced electron transport and coupled

phosphorylation reactions mediated by isolated chloroplasts has been studied extensively (11) and used as a means to explain the mechanism of action of many structurally diverse herbicides known to act as photosynthetic inhibitors (7). Electron transport and photophosphorylation were found to be inhibited by 1,2,4- and 1,3,4-thiadiazole derivatives in assays with isolated chloroplasts (5,15,17). The purpose of this study was to examine the effects of buthidazole and tebuthiuron on the electron transport chain of isolated spinach chloroplasts and to locate the site of the inhibition by segmenting the photosynthetic electron transport pathwy.

MATERIALS AND METHODS

Chloroplast isolation. Chloroplasts were isolated from commercial spinach (Spinacea oleracea L.) obtained from a local market. Leaves were washed with cold distilled water and ground in a Waring blendor for 5 sec in a medium containing 0.3 M NaCl, 30 mM tricine-NaOH buffer (pH 7.8), 3 mM $MgCl_2$, and 0.5 mM EDTA. The homogenate was filtered through eight layers of cheesecloth and the chloroplasts were sedimented at 2500 g for 2 min. The chloroplast pellet was then resuspended in a medium consisting of 0.2 M sucrose, 5 mM HEPES-NaOH buffer (pH 7.4) and 2 mM $MgCl_2$. After a 60-sec centrifugation at 2000 g to remove cell debris, the chloroplasts were centrifuged again (3000 g for 3 min) and finally suspended in a few milliliters of the above suspending medium. Chlorophyll content was determined spectrophotometrically by the method of Arnon (3). All operations were conducted at 0 to 5 C temperature.

Hydroxylamine-treatment of isolated chloroplasts. In the assay of electron transport from catechol to methylviologen the chloroplast suspensions used, were washed with hydroxylamine in order to eliminate flow of electrons from water to methylviologen. Hydroxylamine treatment of chloroplasts was performed according to the method of Izawa and Ort (9). Two ml of chloroplast

stock suspension prepared as described in the previous paragraph were added to 20 ml of a freshly prepared medium containing 0.2 M sucrose, 5 mM HEPES-NaOH buffer (pH 7.4), 2 mM $MgCl_2$, 5 mM NH_2OH and 1 mM EDTA. The mixture was allowed to stand at room temperature (22 C) for 20 min, then diluted with cold, NH_2OH-free suspending medium, and centrifuged at 4000 g for 5 min at 0 C. The chloroplasts were washed twice by centrifugation (4000 g for 5 min, 0 C) with a large volume of the suspending medium to remove NH_2OH and EDTA, and finally suspended in NH_2OH-free suspending medium.

Electron transport assays. Artificial or unnatural electron acceptors and donors have been frequently used in studies of partial reactions of the photosynthetic electron transport mediated by isolated chloroplasts (12,16). In this study uncoupled electron transport from water to ferricyanide and photosystem II-mediated electron transport were assayed spectrophotometrically by recording the rate of ferricyanide reduction at 420 nm. Uncoupled electron transport from water to methylviologen, photosystem I-mediated electron transport and whole chain electron transport from catechol to methylviologen were assayed as O_2 uptake resulting from aerobic reoxidation of reduced methylviologen. A membrane-covered Clark-type electrode was used for these O_2 assays. In all assays light for illumination of the chloroplast preparations was provided by the 500-watt incandescent lamp of a slide projector with a 1-liter round bottomed flask with diluted $CuSO_4$ acting as condenser and heat filter. The light was then passed through a broad band red glass filter (transmission greater than 600 nm) before it impinged on the reaction cuvette. The reaction conditions in each of the assays were as described in Tables I through IV. In all assays the reaction volume was made up to 2.0 ml with distilled water and the reaction temperature was 18 C. Analytical grade buthidazole (100% pure) and tebuthiron (99% pure) were used at concentrations 0, 0.05, 0.5, 5, and 500 μM.

RESULTS AND DISCUSSION

The effects of buthidazole and tebuthiuron on uncoupled electron transport assayed in two ways are shown in Table I. Both compounds at concentrations 0.5 μM and higher inhibited electron transport from water to ferricyanide or methylviologen very strongly (Table I). The inhibitions by the concentrations of 0.05 and 0.5 μM of both herbicides were progressive, indicating a dependence of the ractions on time. Thus preincubation of the reaction mixture with the herbicide in the dark was necessary before initiation of the light reaction. Therefore, electron transport rates for the reactions containing 0.05 and 0.5 μM herbicidal concentrations correspond to reactions preincubated for 5 min in the dark before exposure of the chloroplast preparations to the light.

Photosystem II-mediated transfer of electrons from water to oxidized diaminodurene, with DBMIB acting as block of photosystem I, was also inhibited very strongly by both herbicides at concentrations 0.5 μM or higher (Table II). The inhibition levels obtained with buthidazole and tebuthiuron at 0.05 μM were 34 and 37% respectively.

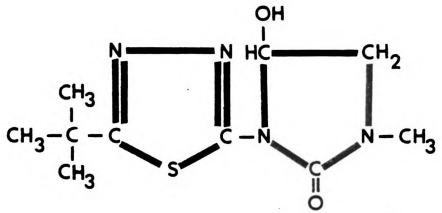
Inhibition of photosystem I-mediated electron transport from ascorbic acid /diaminodurene to methylviologen, with DCMU acting as block of photosystem II, was insignificant with both herbicides at any concentration examined (Table III). This suggests that both buthidazole and tebuthiuron do not inhibit electron transport through photosystem I.

Data presented in Tables I, II, and III indicate that the inhibition of electron transport by both buthidazole and tebuthiuron is primarily at the reducing side of photosystem II, between Q, the unknown primary electron acceptor for photosystem II, and plastoquinone. Thus the site of buthidazole and tebuthiuron inhibition of photosynthesis appears to be the same or very near the site of action of diuron and atrazine (7). This is

not altogether suprising in that the two herbicides tested here are, like diuron, substituted ureas.

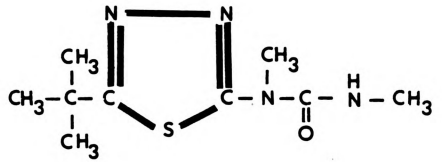
Finally, examination of whole chain of electron transport from ascorbate/catechol to methylviologen in NH_2OH-treated chloroplasts indicated that again electron transport was strongly inhibited by both herbicides (Table IV). However, the inhibition levels caused by both herbicides in this assay were somewhat less than those obtained in the uncoupled electron transport from water to methylviologen (Tables I and IV). Thus the inhibition levels by buthidazole and tebuthiuron at 0.5 μM were 50 and 47%, respectively, as compared to the 89% inhibition levels of the uncoupled electron transport by the same concentration of both herbicides. Since catechol is an electron donor at the oxidizing side of photosystem II, between water and chlorophyll a_{680}, these last results indicate that there might be a small inhibition of the mechanism of water oxidation by both herbicides. This possibility of a secondary site of inhibition on the oxidizing side of photosystem II has also been reported by York and Arntzen (17) who came to the same conclusion on the basis of fluorescence measurements of the effect of buthidazole on electron transport reactions of isolated pea chloroplasts. However, it is also possible that the lower levels of inhibition obtained in the last study might be a consequence of some nonbiological photo-oxidation of catechol or ascorbate, which is not inhibited by the herbicides.

In conclusion, both buthidazole and tebuthiuron inhibited photosynthetic electron transport in vitro. This inhibition was primarily at the reducing side of photosystem II with a small inhibition of the mechanism of water oxidation by both herbicides.



3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone

buthidazole



N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea

tebuthiuron

Figure 1. Chemical structures of the herbicides buthidazole and tebuthiuron

Table I. Effects of buthidazole and tebuthiuron on uncoupled electron transport in illuminated spinach chloroplasts.

Reaction conditions:

50 mM tricine-NaOH buffer (pH 8.0), 2 mM $MgCl_2$, 0.2 M sorbitol, 0.5 mM ferricyanide or 0.5 mM methylviologen, 2 µg/ml gramicidin, and 15 or 7.5 µg of chlorophyll per reaction mixture.

Herbicide concentration (µM)	Photosynthetic reduction of ferricyanide[1]	% Inhibition	Photosynthetic methyl-viologen-mediated O_2 uptake[2]	% Inhibition
Control	1177	0	275	0
Buthidazole				
0.05	941	20	130	53
0.5	102	91	29	89
5	18	98	11	96
500	10	99	7	98
Tebuthiuron				
0.05	906	23	117	58
0.5	112	90	30	89
5	32	97	13	95
500	14	99	7	98

[1] Data expressed as µ moles of ferricyanide reduced/hr/mg of chlorophyll.

[2] Data expressed as µ moles of O_2 consumed/hr/mg of chlorophyll. To compare with electron transport rates in ferricyanide reduction values must be multiplied by 4.

Table II. Effects of buthidazole and tebuthiuron on photosystem II-mediated electron transport in illuminated spinach chloroplasts.

Reaction conditions:

50 mM tricine-NaOH buffer (pH 8.0), 2 mM $MgCl_2$, 0.2 M sorbitol, 2 mM ferricyanide, 0.5 mM diaminodurene, 0.5 μM DBMIB, and 15 μg of chlorophyll per ml of reaction mixture.

Herbicide concentration (μM)	Buthidazole-treated		Tebuthiuron-treated	
	Electron transport rate[1]	% Inhibition	Electron transport rate[1]	% Inhibition
Control	798	0	798	0
0.05	525	34	500	37
0.5	148	82	176	78
5	18	98	37	95
500	0	100	0	100

[1] Data expressed as μ moles of ferricyanide reduced/hr/mg of chlorophyll.

Table III. Effects of buthidazole and tebuthiuron on photosystem I-mediated electron transport in illuminated spinach chloroplasts.

Reaction conditions:

50 mM tricine-NaOH buffer (pH 8.0), 2 mM $MgCl_2$, 0.2 M sorbitol, 0.1 mM methylviologen, 2.5 mM diaminodurene, 2.5 mM ascorbate, 1.5 µM DCMU, and 7.5 µg of chlorophyll per ml or reaction mixture.

Herbicide concentration (µM)	Buthidazole-treated Electron transport rate[1]	% Inhibition	Tebuthiuron-treated Electron transport rate[1]	% Inhibition
Control	1165	0	1165	0
0.05	1101	5	1065	9
0.5	1225	-5	1191	-2
5	1264	-8	1176	-1
500	1230	-6	1187	-2

[1] Data expressed as µ moles of O_2 consumed/hr/mg of chlorophyll. The values given must be multiplied by a factor of between 1 and 2, depending on the endogenous superoxide dismutase and catalase activities to obtain a measure of the true electron transport in the chloroplasts (13).

Table IV. Effects of buthidazole and tebuthiuron on electron transport in hydroxylamine-treated and illuminated spinach chloroplasts.

Reaction conditions:

50 mM tricine-NaOH buffer (pH 8.0), 2 mM $MgCl_2$, 0.2 M sorbitol, 0.5 mM ascorbate, 0.5 mM catechol, 0.5 mM methylviologen, 2 µg/ml gramicidin, and 7.5 µg of chlorophyll per ml of reaction mixture.

Electron donor	Herbicide concentration (µM)	Buthidazole-treated Electron transport rate	Buthidazole-treated % Inhibition	Tebuthiuron-treated Electron transport rate	Tebuthiuron-treated % Inhibition
H_2O	--	282[1]	--	282[1]	--
H_2O (NH_2OH tmt)	--	30	--	30	--
Asc/Cat. (NH_2OH tmt)	Control	137[2]	0	137[2]	0
"	0.05	116	15	120	12
"	0.5	69	50	73	47
"	5	43	69	44	68
"	500	25	82	27	80

[1] Data expressed as µ moles of O_2 consumed/hr/mg of chlorophyll where values must be multiplied by 4 to give electron transport rates.

[2] Data also expressed as µ moles of O_2 consumed/hr/mg of chlorophyll but values must be multiplied by 2 to give electron transport rates.

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CHAPTER 6

Physiological Effects of Buthidazole on Corn (Zea mays L.) Redroot Pigweed (Amaranthus retroflexus L.), Alfalfa (Medicago sativa L.), and Quackgrass [Agropyron repens (L.) Beauv.]

ABSTRACT

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) at concentrations of 10^{-6} to 10^{-4} M did not affect germination of corn (Zea mays L., 'Pioneer 3780'), redroot pigweed (Amaranthus retroflexus L.), alfalfa (Medicago sativa L.,'Vernal'), and quackgrass [Agropyron repens (L.) Beauv.] seeds. Stressing the seeds obtained from mature corn plants treated either preemergence or preplant incorporated with buthidazole at several rates by accelerated aging and cold treatments further indicated that this herbicide did not affect germination. Total photosynthesis and dark respiration of corn plants 12 days after preemergence application and of redroot pigweed, alfalfa, and quackgrass plants after postemergence application of buthidazole at several rates were measured with an infrared CO_2 analyzer. The results suggested that buthidazole was a rapid inhibitor of photosynthesis of the sensitive redroot pigweed and quackgrass plants, with less effect on corn and alfalfa. Buthidazole did not affect respiration of the examined species except for a transitory increase in corn and alfalfa 12 days after preemergence or 4 h after postemergence treatment with buthidazole at 0.56 or 1.12 and 2.24 kg/ha, respectively. A long-term inhibition of quackgrass respiration 96 h after treatment with buthidazole at 1.12 and 2.24 kg/ha was also evident.

INTRODUCTION

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) has shown potential as a preemergence herbicide for selective weed control in corn (1). Applied at low rates

ranging from 0.28 to 0.56 kg/ha, it controls a wide spectrum of weeds. In established alfalfa, buthidazole applied postemergence at 1.12 kg/ha during dormancy effectively controls quackgrass, a serious weed problem in alfalfa (1).

Inhibition of germination, photosynthesis, and respiration have frequently been cited as potential modes of herbicidal action (2,4,6).

The action and selectivity of this herbicide were studied using seedling corn, a crop tolerant to low preemergence application rates of buthidazole, and seedling redroot pigweed, a susceptible weed in the first study. In the second study, buthidazole was applied to alfalfa as the tolerant crop plant and to quackgrass as the susceptible weed as postemergence application to dormant plants.

The purpose of this study was to examine a) effects of buthidazole on germination of corn, pigweed, alfalfa, and quackgrass seeds; b) effects on viability and vigor of corn seeds obtained from plants treated with buthidazole; and c) effects of buthidazole on total photosynthesis and dark respiration of corn and pigweed, following preemergence application, and of alfalfa and quackgrass, at various time periods after postemergence application.

MATERIALS AND METHODS

Germination studies. Twenty seeds of corn (Zea mays L., 'Pioneer 3780') and quackgrass [Agropyron repens (L.) Beauv.] and fifty seeds of alfalfa (Medicago sativa L., 'Vernal') and redroot pigweed (Amaranthus retroflexus L.) were placed in 'Petri' dishes whose bottoms were covered with Whatmann #1 filter paper. Ten milliliters of herbicide solution, pH 6.8, containing 0, 10^{-6}, 10^{-5}, and 10^{-4} M of technical buthidazole (95% purity) were placed in the 'Petri' dishes and the seeds germinated in an incubator at 25 C in the dark. An additional 10 ml of the respective herbicide solution was

added 4 days later to keep the paper moist. After 7 days, germinated seeds were counted and the results expressed as percent germination. Data presented are the means of two experiment with three replications per experiment. The data were analyzed for variance followed by Duncan's multiple range test to separate the means.

Viability and vigor studies of corn seeds obtained from buthidazole-treated corn fields. Seeds harvested from 'Pioneer 3780' corn plants treated in the field with 0, 0.28, 0.56, 0.84, and 1.12 kg/ha of buthidazole applied preemergence or preplant incorporated were obtained from Velsicol Chemical Corporation, Chicago, Illinois. These seeds were analyzed for percent germination and vigor under various environmental conditions as follows:

a) Standard germination test. A standard, warm germination test (5) was run on two 100-seed lots from each sample. The seeds were placed on moist paper towels covered with waxed paper at 25 C for 7 days. Only normal seedlings were recorded. The results were expressed as percent germination.

b) Accelerated aging test. In the accelerated aging stress test (5), two 100-seed lots from each sample were subjected to 42 C for 3 days of 100% relative humidity. They were then transferred to conditions of the standard germination test (25 C, 7 days) and the number of normal seedlings reported as percent germination.

c) Cold treatment. The cold test (5) was performed by placing the seeds on moist unsterilized soil mixture (2/3 greenhouse soil, 1/3 vermiculite), 50 seeds per 473-ml waxed cups, under growth chamber conditions at 10 C for 5 days. The conditions of the growth chamber were then changed to 16-h day at 30 C and 8-h night at 20 C for 6 days. Percent germination, emergence, and seedling height were recorded. In all cases the data were analyzed for variance followed by Duncan's multiple range test to separate the means.

Photosynthesis and respiration studies

Plant material. Five 'Pioneer 3780' corn seeds and ten pigweed seeds were planted into greenhouse soil (1:1:1 soil, sand, peat) in 946-ml waxed cups. Buthidazole was applied preemergence at rates 0, 0.56 and 1.12 kg/ha. After planting, the cups were placed in a greenhouse with temperature ranging from 20 C at night to 33 C during the day. After 12 days photosynthesis and respiration were measured. However, the sensitive pigweed plants died so quickly after emerging from the soil surface that to examine the effect of buthidazole on pigweed photosynthesis we applied buthidazole post-emergence on pigweed plants 25 cm tall.

'Vernal' alfalfa seeds were planted into greenhouse soil in 54 x 36 cm wooden boxes and grown to maturity under greenhouse conditions. Then they were cut to 6 cm, allowed to regrow to 12 cm and placed outdoors during the winter of 1978. Then they were transplanted one plant per 946-ml cup and placed in a greenhouse for acclimation and growth. When the plants reached the height of 25 cm, they were treated with buthidazole and used for the photosynthesis and respiration measurements.

Twenty quackgrass seeds were planted 2.0 cm deep into greenhouse soil in 946-ml waxed food cups and were allowed to grow to maturiy under greenhouse conditions. Then they were cut to 6 cm in height, allowed to regrow to 12 cm, and placed in a controlled environment chamber at 0-5 C for 2 weeks of acclimation. Then the cups were returned to greenhouse conditions and the plants allowed to attain a height of 25-30 cm for use in this study.

Measurement of total photosynthesis and dark respiration. Total photosynthesis and dark respiration of all plant species were measured with an infrared CO_2 analyzer (3,7) in an open air flow system at a slow rate of 500 cm^3/min. The plants, after reaching the aforementioned heights, were placed inside a clear cylinder located in the interior of a growth chamber.

The cylinder was sealed and the lights were turned on and off to create appropriate conditions for measuring total photosynthesis and dark respiration, respectively. The environmental conditions of the growth chamber were 25 ± 1 C day and night temperature and light intensity of 21 Klux or 280 microeinsteims/m^2/sec energy. The measurements were recorded as CO_2 uptake (total photosynthesis) and CO_2 evolution (dark respiration). Following preemergence application of buthidazole in corn, photosynthesis and respiration of corn plants were measured 12 days after treatment. The leaf area of the measured plants was also recorded and the results were expressed as mg $CO_2/dm^2/h$ as reported by Sestak et al. (7). For the postemergence treatment of buthidazole, photosynthesis and respiration were made prior to treatment (original measurements) and then 4 and 24 hours later for redroot pigweed and 4, 24, 48, and 96 hours after treatment for alfalfa and quackgrass plants. The results were expressed as the percentage of the original photosynthetic and respiratory measurements. Redroot pigweed plants were treated with 0, 0.28, 0.56, 0.84, and 1.12 kg/ha of buthidazole, and alfalfa and quackgrass plants were treated with 0, 1.12 and 2.24 kg/ha of the herbicide. In all cases, buthidazole was formulated as a 50% wettable powder and was applied with a link belt sprayer at 2.1 kg/cm^2 pressure in 935 L/ha spray volume.

All data presented are the means of two experiments with two replications per experiment. The data were analyzed for variance followed by Duncan's multiple range test to separate the means.

RESULTS AND DISCUSSION

Buthidazole at concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M did not have any apparent effects on the germination of seeds of corn, redroot pigweed, alfalfa, and quackgrass (Table 1). The redroot pigweed had a low

germination percentage, but this was not the result of treatment with buthidazole since non-treated seeds also germinated poorly.

Extensive testing involving the use of accelerated aging and cold treatment to stress corn seeds during germination might possibly reveal effects of the herbicide that would not show up under normal conditions. However, these tests did not indicate any buthidazole effect on germination (Tables 2 and 3). Seeds for the experiments had been harvested from corn plants treated either preemergence or preplant incorporated with buthidazole at rates 0, 0.28, 0.56, 0.84, and 1.12 kg/ha. In both cases, exposure of seeds to heat stress did not give percent germination values different from those of the regular test. However, exposure of seeds to cold treatment often gave lower germination percentages. There were no significant differences between the germination values of seeds obtained from treated and non-treated plants under the same tests (regular, accelerated aging, and cold germination tests). Percent emergence and seedling height did not show any significant differences between control and herbicide-treatment (Tables 2 and 3).

The influence of buthidazole on total photosynthesis and dark respiration of corn, receiving preemergence application, and redroot pigweed, alfalfa, and quackgrass, all receiving postemergence application, is shown on Tables 4 through 9. Buthidazole appeared to be a rapid photosynthetic inhibitor, acting as early as 4 hours after postemergence application (Tables 5,6,7). However, photosynthesis of corn, 12 days after preemergence application, was not affected by buthidazole at the rate of 0.56 kg/ha, whereas buthidazole at 1.12 kg/ha decreased the photosynthetic rate significantly (Table 4). Following postemergence application, total photosynthesis of redroot pigweed was markedly inhibited by any rate of

buthidazole examined as early as 4 hours after treatment (Table 5). In the second study, total photosynthesis of alfalfa and quackgrass was significantly inhibited very early following postemergence application of buthidazole even at the rate of 1.12 kg/ha (Tables 6 and 7). The time of application may be important for the selective performance of buthidazole in the alfalfa-quackgrass system. For technical reasons in measuring photosynthesis, the plants were allowed to grow to a height of 25 to 30 cm, providing a leaf area greater than in established alfalfa during or right after dormancy. The time of application may be of great significance under field conditions.

The influence of buthidazole on respiration of corn (Table 4), redroot pigweed (Table 5), alfalfa (Table 8), and quackgrass (Table 9) did not appear to be related to the main herbicidal action of the substance. However, it is of interest to note the significant increase in corn respiration 12 days after preemergence treatment with 0.56 kg/ha and in alfalfa respiration 4 hours after postemergence treatment with 1.12 and 2.24 kg/ha (Tables 4 and 8). This burst of CO_2 release was transitory, and levels dropped to normal very rapidly 24 hours after treatment in the case of alfalfa (Table 8). This transitory increase of respiration, observed only in corn and alfalfa, might be an indication of rapid metabolism of buthidazole in these tolerant crop plants. A significant long-term inhibitory effect of buthidazole on quackgrass respiration was detected 96 hours after treatment with 1.12 and 2.24 kg/ha (Table 9). Since this effect was observed only at 96 hours after treatment and not earlier, and furthermore it was observed only in quackgrass, inhibition of respiration did not seem to be a means by which buthidazole exerted its primary mode of action.

In conclusion, the mode of action of buthidazole appears to be a strong and rapid inhibition of photosynthesis with germination of all tested species unaffected.

Table 1. The effect of buthidazole on germination of four plant species*

Buthidazole (Molar Concentration)	Corn	Redroot Pigweed	Alfalfa	Quackgrass
		(percent germination)		
0	100 a	45.0 a	78.3 a	73.3 a
10^{-6}	100 a	41.8 a	83.3 a	76.6 a
10^{-5}	100 a	41.6 a	80.0 a	76.6 a
10^{-4}	100 a	43.6 a	85.0 a	68.3 a

* Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test. Percent values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 2. Viability and vigor of 'Pioneer 3780' corn seeds obtained from plots treated with pre-emergence applied buthidazole*.

	Standard Germination Test	Accelerated Aging Test	Cold Test		
Buthidazole	Germination	Germination	Germination	Emergence	Height
(kg/ha)	(%)	(%)	(%)	(%)	(cm)
0	97.5 a	93.2 a	72.5 a	79.1 a	6.0 a
0.28	97.4 a	95.0 a	69.1 a	76.6 a	6.3 a
0.56	97.6 a	92.5 a	74.1 a	87.4 a	5.6 a
0.84	97.9 a	95.7 a	75.8 a	85.0 a	5.4 a
1.12	95.9 a	94.5 a	72.1 a	84.9 a	6.1 a

* Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test. Percent values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 3. Viability and vigor of 'Pioneer 3780' corn seeds obtained from plots treated with pre-plant incorporated buthidazole*.

	Standard Germination Test	Accelerated Aging Teat	Cold Test		
Buthidazole	Germination	Germination	Germination	Emergence	Height
(kg/ha)	(%)	(%)	(%)	(%)	(cm)
0	97.7 a	96.2 a	86.6 ab	88.3 a	6.1 a
0.28	96.0 a	95.5 a	67.5 a	84.9 a	4.5 a
0.56	98.2 a	96.5 a	86.6 ab	88.3 a	6.7 a
0.84	97.2 a	93.7 a	92.4 b	94.1 a	6.7 a
1.12	98.5 a	96.0 a	89.1 ab	92.4 a	6.8 a

* Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test. Percent values less than 15% or greater than 85% were transformed to arcsine values for analysis of variance.

Table 4. The effect of buthidazole on corn total photosynthesis and dark respiration 12 days after preemergence application*.

Buthidazole	12 days after treatment	
	Photosynthesis+	Respiration±
(kg/ha)	(mg $CO_2/dm^2/hr$)	
0	41 b	9 a
0.56	44 b	17 b
1.12	33 a	11 a

* Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

\+ CO_2 uptake

± CO_2 evolution

Table 5. The effect of buthidazole on redroot pigweed total photosynthesis and dark respiration at various time intervals after postemergence application*.

	Hours after postemergence treatment			
	4		24	
Buthidazole	Photosynthesis^{+}	Respiration$^{\pm}$	Photosynthesis	Respiration
(kg/ha)	(ppm of CO_2 as percent of the original values)			
0	121 d	100 a	150 b	124 a
0.28	48 c	103 a	0 a	80 a
0.56	16 b	109 a	0 a	125 a
0.84	12 ab	98 a	0 a	92 a
1.12	2 a	87 a	0 a	95 a

* Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

$^{+}$ CO_2 uptake

$^{\pm}$ CO_2 evolution

Table 6. The effect of buthidazole on 'Vernal' alfalfa total photosynthesis at various time intervals after postemergence application*.

Buthidazole	Hours after treatment			
	4	24	48	96
(kg/ha)	(ppm of CO_2 uptake as % of the original values)			
0	117 b	149 b	185 c	190 c
1.12	7 a	9 a	10 a	21 a
2.24	0 a	0 a	0 a	0 a

* Means whithin rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 7. The effect of buthidazole on quackgrass total photosynthesis at various time intervals after postemergence application*.

Buthidazole	Hours after treatment			
	4	24	48	96
(kg/ha)	(ppm of CO_2 uptake as % of the original values)			
0	94 b	117 c	124 c	99 b
1.12	9 a	0 a	0 a	0 a
2.24	8 a	0 a	0 a	0 a

* Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 8. The effect of buthidazole on dark respiration of 'Vernal' alfalfa at various time intervals after postemergence application*.

Buthidazole	Hours after treatment			
	4	24	48	96
(kg/ha)	(ppm of CO_2 evelution as % of the original values)			
0	115 a	108 a	112 a	110 a
1.12	169 b	140 a	132 a	137 a
2.24	189 b	136 a	115 a	117 a

* Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

Table 9. The effect of buthidazole on quackgrass dark respiration at various time intervals after postemergence application*.

Buthidazole	Hours after treatment			
	4	24	48	96
(kg/ha)	(ppm of CO_2 evelution as % of the original values)			
0	88 abc	114 cd	113 cd	126 d
1.12	99 bcd	111 bcd	95 abcd	82 ab
2.24	91 abc	92 abc	83 abc	68 a

* Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

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CHAPTER 7

Some Effects of Buthidazole on Corn (Zea mays L.) Photosynthesis, Respiration, Anthocyanin Formation, and Leaf Ultrastructure

ABSTRACT

The effect of the herbicide buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) on photosynthesis, respiration, anthocyanin formation, and leaf ultrastructure of corn (Zea mays L., var. Pioneer 3780) was studied following pre- or post- emergence applications. Total photosynthesis and dark respiration were measured with an infrared CO_2 analyzer in an open air flow system 12, 18, and 24 days after preemergence treatment with 0, 0.56, 1.12, and 2.24 kg/ha of buthidazole. The 0.56 and 1.12 kg/ha preemergence treatments had no effect on total corn photosynthesis even 24 days after treatment, whereas buthidazole at 2.24 kg/ha inhibited photosynthesis as early as 12 days. Total photosynthesis and dark respiration were also measured in whole plants, 30 cm tall, before herbicide application and 4, 24, 48, and 96 h after postemergence treatment with buthidazole at 0, 0.28, 0.56, 0.84, and 1.12 kg/ha. Following postemergence treatment, buthidazole inhibited total photosynthesis at any rate examined as early as 4 h after treatment. Neither pre- or postemergence buthidazole applications influenced respiration with the exception of a transitory increase caused by 0.56 kg/ha 12 days after preemergence treatment and by 0.84 and 1.12 kg/ha 4 h after postemergence treatment. Transmission electron micrographs revealed that buthidazole applied postemergence at 0.28 and 1.12 kg/ha reduced or prevented the accumulation of starch in bundle sheath chloroplasts as early as 24 h after treatment. Ultrastructural disruptions in some mesophyll chloroplasts of treated corn plants were also evident. Preemergence application of buthidazole at rates of 0.28, 0.42, 0.56, and 1.12 kg/ha inhibited anthocyanin formation

indicating an alteration in corn metabolism.

INTRODUCTION

Photosynthesis and respiration have been repeatedly reported to be affected by many herbicides (3,13,15). Electron microscope studies have served as a useful tool in studying the mode of action of herbicides (1).

Derivatives of 1,3,4-thiadiazoles exhibit insecticidal (4) and herbicidal (14) activities. Buthidazole, a derivative of 1,3,4-thiadiazole (Figure 1), is currently marketed as a herbicide for industrial weed control. Its potential for selective weed control in corn, using preemergence or early postemergence applications at low rates (0.28 to 0.56 kg/ha), is the object of current research (2). High rates are phytotoxic even to corn, making corn a suitable species for the study of buthidazole selectivity and mode of action. Our objectives were to study the mode of buthidazole action by examining the effect of buthidazole on photosynthesis, dark respiration, anthocyanin formation, and leaf ultrastructure in corn.

MATERIALS AND METHODS

Plant material and herbicide application. 'Pioneer 3780' corn was seeded five seeds per 946-ml pot in a 1:1:1 volume mixture of soil, sand, and peat and placed in the greenhouse at 25 ± 3 C. Buthidazole was applied preemergence at 0, 0.56, 1.12, and 2.24 kg/ha for the photosynthesis and respiration studies and at 0, 0.28, 0.42, 0.56, and 1.12 kg/ha for the anthocyanin study. In separate experiments when corn plants were 30 cm tall, at 20 days of age, buthidazole was applied postemergence at rates 0, 0.28, 0.56, 0.84, and 1.12 kg/ha for photosynthesis and respiration measurements. In all cases the herbicide was formulated as a 50% wettable powder, and it was sprayed with a link belt sprayer at 2.1 kg/cm^2 pressure in 935 L/ha spray volume.

Photosynthesis and respiration measurements. Total photosynthesis and dark respiration were measured with an infrared CO_2 analyzer in an open gas flow

system operated at 500 cm^3/min. After acclimation to insure open stomata the plants were placed in a sealed cylinder inside a growth chamber with conditions of 25 ± 1 C day and night temperature and light intensity of 21 Klux or 280 $\mu E/m^2$/sec energy. The measurements were over the period of 1 hour as ppm of CO_2 uptake (total photosynthesis) and as ppm of CO_2 evolution (dark respiration). Following the preemergence application of buthidazole, total photosynthesis, dark respiration, and leaf area were measured 12, 18, and 24 days after treatment. The results were expressed as mg CO_2/dm^2/h by means of the following formula (17).

$$mgCO_2/dm^2/h = \frac{\text{Flow rate (L/min)} \times \Delta CO_2 \text{ (ppm)} \times \frac{273\ K}{298\ K} \times \frac{44 g CO_2/mole}{22.4\ L/mole} \times 10^3 \frac{mg}{g} \times 60 \frac{min}{h}}{\text{Leaf area } (m^2) \times 100 \frac{dm^2}{m^2} \times 10^6 \frac{\mu l}{L} \text{ (ppm)}}$$

In this equation ΔCO_2 refers to the difference in CO_2 content of the inlet and outlet gas streams.

For the postemergence treatment of buthidazole, photosynthesis and respiration measurements were made before treatment and then 4, 24, 48, and 96 h after treatment. The results are expressed as the percentage of the pretreatment photosynthetic and respiratory measurements.

Data presented are the means of two experiments with two replications per experiment for all studies. The data were analyzed by analysis of variance in a two-way factorial design with factor A as the herbicide rate and factor B as the time period after treatment with the herbicide. Mean separation was by Duncan's multiple range test. A student's t-test was also used to compare the values obtained for the treated and non-treated plants at the various time intervals after the postemergence application to those corresponding to the pretreatment measurements.

Transmission electron microscopy (TEM) study. Tissue samples from corn leaves were obtained from an area 6 cm from the tip of the third leaf 24 and 96 h after treatment with buthidazole at rates of 0, 0.28, and 1.12 kg/ha. These tissues were fixed for 2 h at 25 C in 5% (v/v) glutaraldehyde and Sorensen's phosphate buffer, pH 7.2 (10). The material was then washed in the same buffer and fixed for 1 h in 1% (v/v) osmium tetroxide. After washing, the tissues were stained in 0.5% (w/v) aqueous uranyl acetate for 2 h. The material was then dehydrated in ethanol and embedded in Epon-Araldite (6). Thin sections were obtained in an ultramicrotome equipped with a diamond knife. These sections were stained with lead citrate and examined with a Philips 201 TEM at 60 kV.

Anthocyanin extraction. Plants used for this study were grown in a growth chamber with 16-h day and 8-h night under the same conditions as in the photosynthesis studies. Fourteen days after preemergence treatment with buthidazole, the plants were harvested and anthocyanin was extracted as described by Duke et al.(9). The sheaths from the first leaves were ground with 10 ml of cold methanolic HCl (1% HCl) in a mortar and pestle. The extract was centrifuged at 1700 g for 10 min, and the absorbance of the supernatant was measured spectrophotometrically at 525 nm. The values presented are the means of three experiments with five repications per experiment.

RESULTS AND DISCUSSION

Preemergence application of buthidazole at 0.56 and 1.12 kg/ha had no effect on total photosynthesis of corn even 24 days after treatment (Table 1). However, buthidazole at 2.24 kg/ha caused significant inhibition of photosynthesis as early as 12 days after treatment (Table 1). The significant difference observed between the photosynthetic rates at 12

days and 18 or 24 days, even for the control plants, was due to senescence of part of the lower leaves of the older plants 18 and 24 days after treatment.

Following postemergence treatment buthidazole inhibited total corn photosynthesis at any rate examined as early as 4 h after treatment (Table 2). The data indicate that buthidazole applied either pre- or postemergence is a photosynthetic inhibitor and the effect appears dose dependent. Greater time was required to inhibit photosynthesis following preemergence than postemergence buthidazole application due to the time required for germination and development of roots and the emerging shoot (coleoptile) which appear to be important for the uptake of soil applied buthidazole (11).

No effect of buthidazole on dark respiration of corn was evident following either pre- or postemergence applications, with the exception of an increase of the respiratory rates caused by 0.56 kg/ha 12 days after preemergence treatment and by 0.84 and 1.12 kg/ha after postemergence treatment (Tables 3 and 4). This burst of CO_2 release was transitory and levels dropped to normal rapidly (Tables 3 and 4).

Electron micrographs of leaf sections obtained from corn plants 24 and 96 h after postemergence treatment with buthidazole at 0, 0.28, and 1.12 kg/ha are shown in figures 2 and 3. Reduction of the amount of starch synthesized or prevention of its accumulation in bundle sheath chloroplasts of treated corn plants was noticeable 24 h after treatment with 1.12 kg/ha of the herbicide (Figure 2d). Mesophyll chloroplasts appeared swollen 24 h after treatment with 0.28 kg/ha of buthidazole, and ultrastructural disruprions of chloroplast membranes were present 96 h after treatment with 0.28 and 1.12 kg/ha of the herbicide (Figures 3c, 3d, and 3f). However, normal mesophyll chloroplasts were also observed in some

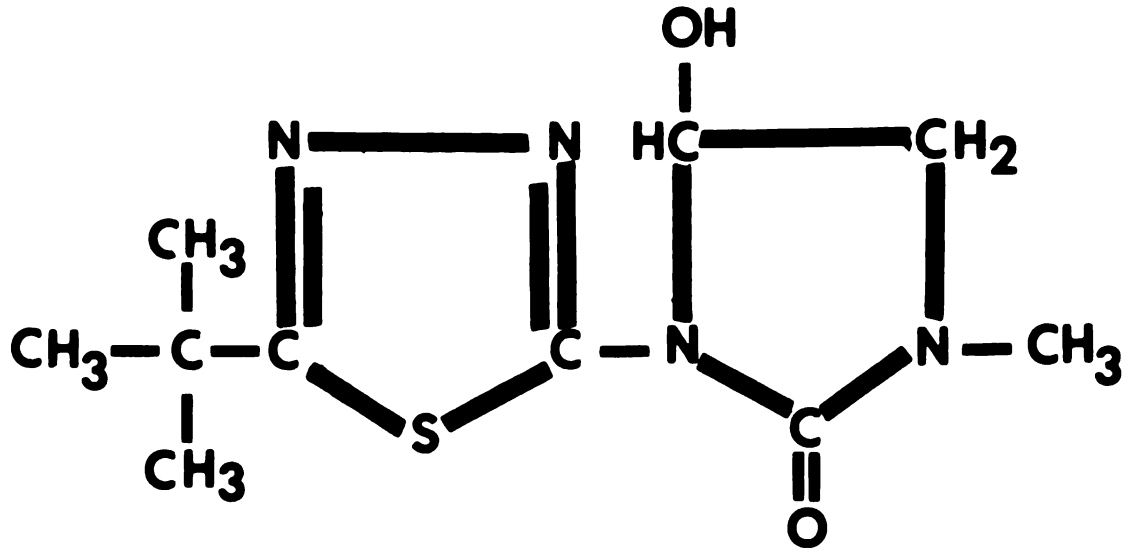
sections obtained 96 h after treatment with this compound (Figure 3e). Swelling of chloroplast thylakoids has been reported to be caused by other herbicides and is often considered as an early stage of chloroplast breakdown (1). No obvious abnormalities due to treatment with this herbicide were observed in mitochondria of treated corn plants (Figure 3b). Thus, the ultrastructural studies support the results obtained from the photosynthesis and respiration measurements, indicating again that buthidazole is a photosynthetic inhibitor. This conclusion is also supported from another study in which buthidazole was found to be a very strong inhibitor of photosynthetic electron transport in isolated spinach chloropalsts, comparable to atrazine and diuron (12).

Preemergence application of buthidazole at rates as low as 0.28 kg/ha inhibited anthocyanin formation in the sheaths of the first leaves in corn, indicating another effect on metabolism (Table 5). Anthocyanins are flavonoid compounds that are nearly always present as glycosides containing most commonly one or two glucose or galactose units attached to the hydroxyl group in the central ring of their molecule. These sugars can be formed from degradation of starch or fat in storage organs during seedling development or from photosynthesis in chlorophyll-containing cells (5,16). Therefore, this effect of buthidazole on anthocyanin formation in corn seems to be a consequence of the effect of buthidazole on corn photosynthesis discussed earlier. Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] and monuron [3-(p-chlorophenyl)-1,1-dimethylurea], two well-known powerful photosynthesis inhibitors, have also been demonstrated to reduce photo-induced levels of anthocyanin (5,7,8) and activity of phenylalanine ammonia lyase (18), respectively. However, light-induced formation of anthocyanin in corn seedlings was found to be independent of photosynthesis in

one study (9), and in our study inhibition of photosynthesis was not evident at rates causing inhibition of anthocyanin formation in the sheaths of corn leaves (Tables 1 and 5). In such a case a possible effect of buthidazole on the activity of phenylalanine ammonia lyase, or other key enzymes in anthocyanin biosynthesis, could serve as a basis to explain the aforementioned effect of buthidazole on anthocyanin formation in corn. Further work is needed to elucidate this point.

In conclusion, buthidazole appears to be a strong inhibitor of photosynthesis and anthocyanin biosynthesis in corn. Buthidazole at low rates stimulated respiration in corn, but the effect was transitory.

Figure 1. Chemical structure and chemical name of the herbicide buthidazole.



3-[5-(1,1-dimethylethyl)-1,3,4- thiadiazol-2-yl]-4- hydroxy-1-methyl-2- imidazolidinone

BUTHIDAZOLE

Figure 2. Electron micrographs of corn bundle sheath chloroplasts from a) Control 96 h after treatment with water and from buthidazole-treated plants; b) and c) 24 and 96 h after treatment at 0.28 kg/ha and d) and e) 24 and 96 h after treatement at 1.12 kg/ha. Bar represents 0.5 µm in a, b, e, 0.2 µm in c, and 0.25 µm in d.

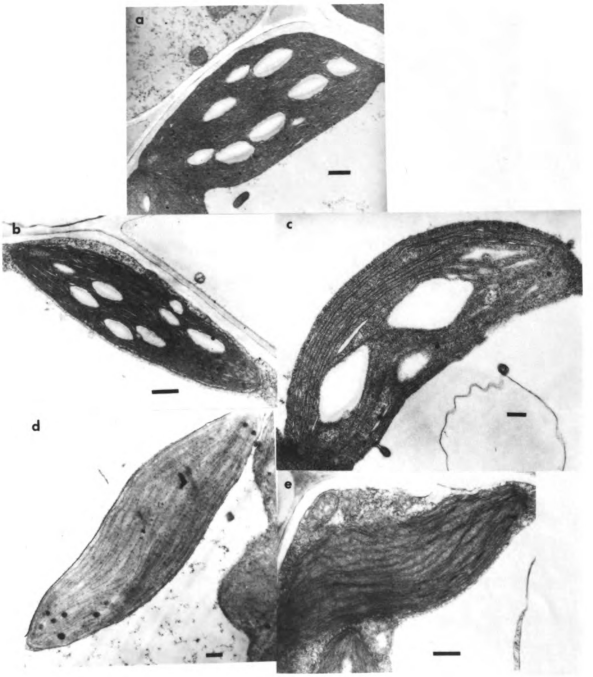
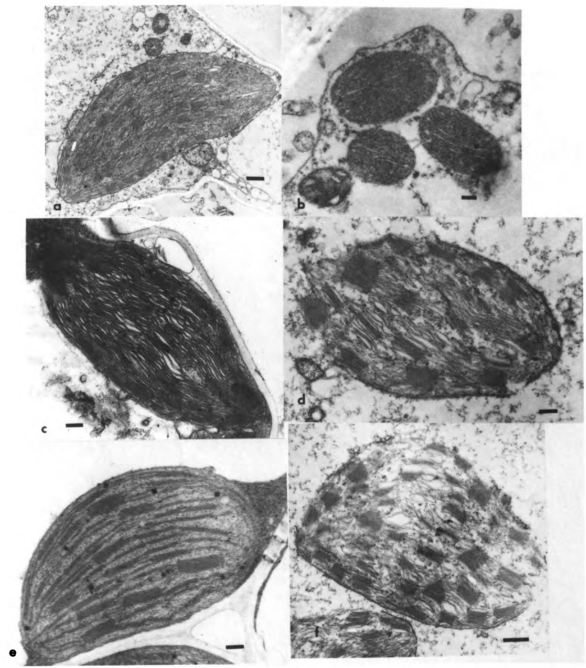

a
b
c
d
e

Figure 3. Electron micrographs of corn mesophyll chloroplasts and mitochondria from a) Control 96 h after treatment with water and from buthidazole-treated plants; b) Mitochondria 96 h after treatment at 1.12 kg/ha, c) and d) 24 and 96 h after treatment at 0.28 kg/ha, and e) and f) 24 and 96 h after treatment at 1.12 kg/ha. Bar represents 0.5 μm in a, f, 0.1 μm in b, and 0.25 μm in c,d, and e.


a
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e
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Table 1. Effect of buthidazole on 'Pioneer 3780' corn total photosynthesis at various time intervals after preemergence application.[a]

Buthidazole	Days after treatment		
	12	18	24
(kg/ha)	(CO_2 uptake as mg $CO_2/dm^2/hr$)		
0	40 fg	26 de	26 de
0.56	45 g	25 cde	23 cde
1.12	33 ef	24 cde	17 bcd
2.24	15 bc	10 ab	2 a

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

Table 2. Effect of buthidazole on 'Pioneer 3780' corn total photosynthesis at various time intervals after postemergence application.[a]

Buthidazole	Hours after treatment			
	4	24	48	96
	(ppm of CO_2 uptake as % of the original values)[b]			
(kg/ha)				
0	118 f	133** g	158** h	152** h
0.28	84* e	53** cd	58** de	55** cd
0.56	71** de	33** b	33** b	29** b
0.84	41** bc	10** a	9** a	9** a
1.12	30** b	7** a	7** a	4** a

[a] Means within rows and columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

[b] Asterisks (* or **) within rows indicate significant differences at the 5% and 1% levels between the means of the measurements conducted at 4, 24, 48, and 96 h after treatment and the original values by student's t-test.

Table 3. Effect of buthidazole on 'Pioneer 3780' corn dark respiration at various time intervals after preemergence application.[a]

Buthidazole	Days after treatment		
	12	18	24
(kg/ha)	(CO_2 evolution as mg $CO_2/dm^2/hr$)		
0	8.3 ab	6.7 ab	8.9 ab
0.56	16.0 c	6.5 ab	9.1 b
1.12	8.5 ab	7.3 ab	6.5 ab
2.24	8.7 ab	5.5 a	7.6 ab

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

Table 4. Effect of buthidazole on 'Pioneer 3780' corn dark respiration at various time intervals after postemergence application.[a]

Buthidazole	Hours after treatment			
	4	24	48	96
(kg/ha)	(ppm of CO_2 evolution as % of the original values)[b]			
0	94 ab	91 ab	102 b	97 ab
0.28	87 ab	78 a	89 ab	76 a
0.56	92 ab	79 a	82 ab	87 ab
0.84	134** c	84 ab	85 ab	88 ab
1.12	144** c	83 ab	94 ab	78 a

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

[b] Asterisks (**) within rows indicate significant differences at the 1% level, between the means of the measurements conducted at 4 h after treatment and the original values by student's t-test.

Table 5. The effect of buthidazole on anthocyanin formation in the sheaths of 'Pioneer 3780' corn leaves 14 days after preemergence application[a].

Buthidazole	Anthocyanin formation
(kg/ha)	(Absorbance at 525 nm)
0	0.559 c
0.28	0.287 b
0.42	0.205 ab
0.56	0.082 a
1.12	0.126 ab

[a] Means within columns with similar letters are not significantly different at the 5% level by Duncan's multiple range test.

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CHAPTER 8

Potential Antidotes Against Buthidazole Injury to Corn (*Zea mays* L.)

ABSTRACT

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) has shown promise for selective weed control in corn (*Zea mays* L.). However, at high rates, injury symptoms were evident in corn seedlings. Greenhouse studies were initiated to test potential antidotes for buthidazole injury to corn seedlings. NA (1,8-naphthalic anhydride) and CDAA (2-chloro-*N*,*N*-diallylacetamide) were the most promising of six chemicals evaluated. The other chemicals tested were R-25788 (2,2-dichloro-*N*,*N*-diallylacetamide), R-29148 (2,2-dimethyl-5-methyl-dichloroacetyloxazolidine), carboxin (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxanthiin) and gibberellin (GA_3). Seven herbicides were also tested for their antagonistic interactions but none offered protection. The herbicides were alachlor [2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetamide], metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide], diethatyl [*N*-(chloroacetyl)-*N*-(2,6-diethylphenyl)-glycine ethyl ester], H-26910 [*N*-(chloroacetyl)-*N*-(2-methyl-6-ethylphenyl)-glycine isopropyl ester], EPTC (*S*-ethyl dipropylthiocarbamate), butylate (*S*-ethyl diisobutylthiocarbamate) plus the antidote R-25788, and trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine). A ratio 1:3 (buthidazole:CDAA) appeared to be optimal for the protection effect. CDAA appeared to be more effective than NA. Since both CDAA and NA offered limited protection but R-25788 did not, this action appears to be through a different mechanism than the one proposed against the thiocarbamate or acetanilide herbicides.

INTRODUCTION

The concept of using herbicide antidotes, introduced by Hoffman in 1962 (12), offers a potential alternative for increasing the margin of selectivity of the currently available herbicides. Three compounds have received commercial interest, NA (1,8-naphthalic anhydride) for protecting corn against thiocarbamate, dithiocarbamate, and acetanilide herbicides (13) and for protecting rice, grain sorghum, and oats against other herbicides (6,15,24). R-25788 (2,2-dichloro-N,N-diallylacetamide) was effective against thiocarbamate injury to corn (23) and has been found effective against perfluidone (1,1,1-trifluoro-N-[2-methyl-4-(phenylsulfonyl)phenyl]methanesulfonamide) (24), barban (4-chloro-2-butynyl m-chlorocarbanilate) (4), and acetanilide (18) injury to corn. Protection offered by R-25788 appears very specific to corn (25). CGA 43089 [α-(cyanomethoximino)-benzonitrile] has shown promise in protecting sorghum against metolachlor (8,22). Numerous other compounds have been tested including R-29148 (2,2-dimethyl-5-methyl-dichloro-acetyloxazolidine) and CDAA (2-chloro-N,N-diallylacetamide), an analog of R-25788 (Figure 1), which were less effective as antidotes against thiocarbamate and acetanilide injury to corn than R-25788 (5,16,19). Gibberellin (GA_3) and carboxin (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxanthiin) also have been reported to decrease injury from herbicides (7,21).

Buthidazole (3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone) is a promising herbicide for selective weed control in corn. However, the margin of selectivity is narrow and buthidazole at rates higher than 0.56 kg/ha may cause injury to corn seedlings following preemergence application (1).

The purpose of this study was to determine whether corn tolerance to buthidazole could be increased using chemicals or herbicides previously reported to have antidote activity.

MATERIALS AND METHODS

Plant material and chemical treatment evaluation. 'Pioneer 3780' corn plants for all studies were grown in greenhouse soil (1:1:1 soil, sand, peat), 5 seeds per 946-ml waxed cups. Four acetanilide (alachlor, metolachlor, diethatyl, H-26910), two thiocarbamate (EPTC and butylate+R-25788) and one dinitroaniline (trifluralin) herbicides were evaluated for their efficacy in protecting corn from buthidazole when applied in combination with buthidazole. These herbicides and the potential antidotes R-25788, CDAA, and GA_3, were sprayed on the soil as formulated emulsifiable concentrates in an oil-in-water emulsion. The formulated emulsifiable concentrate of R-29148 was sprayed in a 50% water-50% ethanol mixture. Buthidazole formulated as a 50% wettable powder and the herbicides and antidotes were sprayed sequentially with a link belt sprayer at 2.1 kg/cm^2 pressure in 935 L/ha spray volume. Buthidazole was applied at 0, 1.12, 2.24 kg/ha in all studies and, in addition, at 0.42, 0.56 and 0.84 kg/ha in two studies. The six antidotes used throughout this study were: R-25788 at 0, 1.12, 2.24, and 5.60 kg/ha, R-29148 at 0, 1.12, and 2.24 kg/ha, gibberellin (GA_3) as 3.91% liquid concentrate at 0 and 1.12 kg/ha, CDAA at 0, 1.12, 2.24, 3.36, 4.48, 5.6 and 6.72 kg/ha, NA and Carboxin at 0 and 0.5% (w/w) seed treatments. The herbicides tested for antagonistic interactions with buthidazole were alachlor at 0, 1.12, 2.24, 3.36, and 5.6 kg/ha, metolachlor at 0, 1.12, 2.24, and 3.36 kg/ha, diethatyl at 0, 0.56, 3.36 and 5.6 kg/ha, H-26910 at 0, 0.56, and 3.36 kg/ha, EPTC at 0, 1.12, and 3.36 kg/ha, butylate+R-25788 at 0, and 2.24 kg/ha, and trifluralin at 0, 0.28 and 0.56 kg/ha. After planting and preemergence application of the chemicals, the cups were placed in a greenhouse with temperature ranging from 20 C at night to 33 C during the day. Twenty or 30 days after planting, the plants were harvested and plant heights and fresh weights were measured. The data are expressed

as the average shoot height in cm per plant per cup and the average shoot fresh weight in g per plant per cup. Data presented are the means of two experiments with five replications per experiment. These data were analyzed fcr variance and Duncan's multiple range test was used to separate the means.

RESULTS AND DISCUSSION

The antidote R-29148 at 2.24 kg/ha offered limited protection against 2.24 kg/ha of buthidazole (Table 1). R-25788 was ineffective at that rate or at 1.12 kg/ha. GA_3 applied at 1.12 kg/ha increased shoot height but not shoot fresh weight, indicating that the plants were taller but still injured (Table 1). CDAA at 1.12 and 2.24 kg/ha offered limited protection to corn injury from buthidazole at 1.12 kg/ha (Table 2). Increasing the ratio of buthidazole to CDAA to 1:3 provided greater protection from CDAA (Table 2).

Increasing the rates of CDAA to 4.48, 5.6, and 6.72 kg/ha did not increase the protection (Table 3). Although the ratio of 1:3 (buthidazole: CDAA) may not be practical, it shows that buthidazole safety to corn can be enhanced chemically.

Data in Table 4 indicate that NA at 0.5% (w/w) also offered limited protection to corn from buthidazole injury at rates of 0.84 and 1.12 kg/ha, whereas carboxin failed to give any protection. Thus, NA, a chemical antidote against a spectrum of herbicides in corn and other grasses (2), also appeared promising against buthidazole injury to corn. CDAA differs from R-25788 only in that it has one less chlorine (Figure 1). This difference appears important in protecting thiocarbamate and acetanilide injury to corn, with R-25788 being superior to CDAA (16,19). A 12:1 ratio of EPTC or butylate to R-25788 was very effective for corn protection (18). R-25788 applied at a 5:1 ratio of antidote to buthidazole, which corresponds to amount of R-25788 60 times greater than that needed to work against

thiocarbamates, failed to protect corn from buthidazole injury (Table 6). The difference in chemistry of R-25788 and CDAA was again evident, however, this time CDAA was active and R-25788 inactive.

Protection of corn from EPTC and other thiocarbamate injury is believed to be the result of an R-25788-mediated increase of the rate of EPTC sulfoxidation followed by subsequent EPTC sulfoxide-glutathione conjugation (3,16,17,20). The moderate antidotal activity of CDAA against EPTC injury to corn may also result from increased EPTC metabolism (16). A similar mode of action has also been proposed for NA which was reported to stimulate EPTC (9) and cisanilide (14) metabolism in corn. To protect corn against buthidazole injury, CDAA and NA may act through a different mechanism than the one proposed for the thiocarbamates or acetanilide herbicides. This hypothesis is partially supported by the finding that ^{14}C buthidazole did not conjugate with ^{3}H-labeled blutathione (GSH) *in vitro* (20). Although this observation does not exclude an enzymatic GSH conjugation of buthidazole *in vivo*, it does suggest that the activity of the responsible glutathione-*S*-transferase was not stimulated by R-25788, CDAA, and NA.

Buthidazole has been reported to act as a strong inhibitor of photosynthesis in corn and other plants (10) blocking photosynthetic electron transport primarily at the reducing site of photosystem II (11). Therefore, the protection of corn injury from buthidazole obtained with the use of NA and CDAA appears to be a successful attempt in antidoting a herbicide affecting photosystem II. Thus far, no one has reported success in antidoting diuron or other herbicides affecting photosystem II (13).

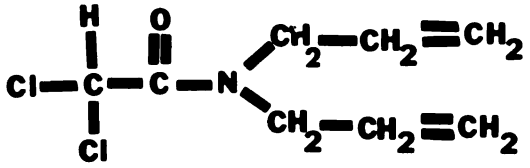
The acetanilide, thiocarbamate, and dinitroaniline herbicides, tested for antagonistic interactions with buthidazole, were not effective in preventing corn injury from buthidazole (Tables 5,6,7). At a herbicide to antidote ratio of 2:3, diethatyl offered slight protection (Table 5).

However, this does not appear promising, as higher rates of diethatyl were phytotoxic to corn (Table 6). York and Slife (26) reported promising antagonistic interactions between buthidazole and acetanilide herbicides on corn, however, the rates of buthidazole and acetanilide herbicides used in their studies were not reported. The data in Table 7 indicate that alachlor at 2.24 kg/ha offered limited protection against 0.56 kg/h a of buthidazole, but no protection was evident against higher rates of buthidazole. Metolachlor and butylate+ R-25788 also failed to offer any protection (Table 7).

In summary, CDAA and NA, although they offered only partial protection, were the most effective antidotes tested against buthidazole injury to corn. The mechanism of CDAA and NA action appeared different from that proposed for these antidotes and R-25788 against thiocarbamate or acetanilide injuries in corn. Perhaps analogs of CDAA, other than R-25788 or R-29148, may offer greater protection against buthidazole.

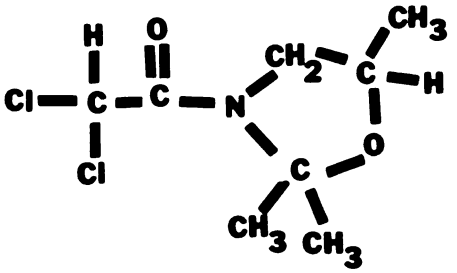
Figure 1. Chemical structures and chemical names of the herbicide antidotes, R-25788, R-29148, CDAA, carboxin, and NA.

R-25788



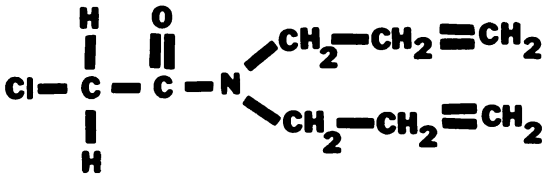
2,2-dichloro-N,N-diallylacetamide

R-29148



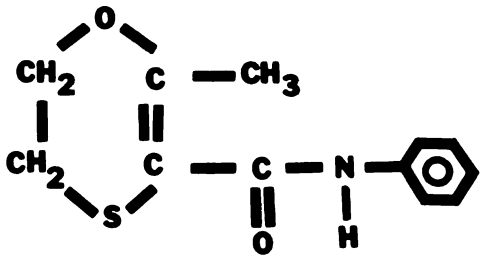
2,2-dimethyl-5-methyl-dichloroacetyloxazolidine

CDAA



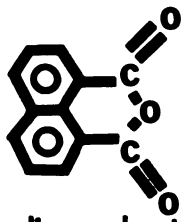
2-chloro-N,N-diallylacetamide

CARBOXIN



2,3-dihydro-carboxanilido-6-methyl-1,4-oxanthiin

NA



1,8-naphthalic anhydride

Table 1. Effects of R-25788, R-29148, and GA_3 on corn injury from buthidazole, 30 days after preemergence treatment[a].

	Buthidazole (kg/ha)	Antidotes	Rate (kg/ha)	Shoot ht (cm/plant)	Shoot fresh wt (g/plant)
Main effects					
(i) Antidotes		none	0.0	44.0 a	2.7 a
		R-25788	2.24	46.1 a	3.0 ab
		R-29148	2.24	43.2 a	2.8 ab
		GA_3	1.12	51.5 b	3.2 b
(ii) Buthidazole	0.0			57.1 c	5.0 c
	1.12			47.3 b	2.9 b
	2.24			34.3 a	0.8 a
Interactions	0.0	none	0.0	56.7 f	5.2 f
	1.12		0.0	45.2 cd	2.4 cd
	2.24		0.0	30.1 a	0.4 a
	0.0	R-25788	2.24	57.8 f	5.3 f
	1.12		2.24	47.5 cde	3.3 de
	2.24		2.24	33.2 ab	0.4 a
	0.0	R-29148	2.24	49.8 de	4.0 e
	1.12		2.24	43.1 c	2.6 d
	2.24		2.24	36.7 b	1.6 bc
	0.0	GA_3	1.12	63.9 g	5.7 f
	1.12		1.12	53.4 ef	3.2 de
	2.24		1.12	37.1 b	0.8 ab

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 2. Effect of CDAA on corn injury from buthidazole, 30 days after pre-emergence treatment[a].

	Buthidazole	CDAA	Shoot ht	Shoot fresh wt
	(kg/ha)	(kg/ha)	(cm/plant)	(g/plant)
Main effects				
(i) CDAA		0.0	44.0 a	2.7 a
		1.12	49.6 b	3.1 ab
		2.24	53.3 c	3.4 bc
		3.36	53.9 c	3.6 c
(ii) Buthidazole	0.0		59.4 c	5.1 c
	1.12		53.3 b	3.5 b
	2.24		37.9 a	1.0 a
Interactions	0.0		56.8 de	5.2 e
	1.12	none	45.0 c	2.4 c
	2.24		30.1 a	0.4 a
	0.0	1.12	58.3 d	4.9 e
	1.12	1.12	52.7 d	3.4 d
	2.24	1.12	37.8 b	0.9 ab
	0.0	2.24	60.9 e	5.1 e
	1.12	2.24	57.5 de	3.9 d
	2.24	2.24	41.4 bc	1.2 ab
	0.0	3.36	61.5 e	5.1 e
	1.12	3.36	57.8 de	4.2 de
	2.24	3.36	42.3 bc	1.6 bc

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 3. Effect of CDAA on corn injury from buthidazole, 30 days after pre-emergence treatment[a].

	Buthidazole (kg/ha)	CDAA (kg/ha)	Shoot ht (cm/plant)	Shoot fresh wt (g/plant)
Main effects				
(i) CDAA		0.0	47.7 a	2.7 a
		4.48	52.7 b	3.0 ab
		5.6	55.7 b	3.5 c
		6.72	55.3 b	3.2 bc
(ii) Buthidazole	0.0		74.2 c	7.1 c
	1.12		53.4 b	2.0 b
	2.24		30.9 a	0.2 a
Interactions	0.0		73.2 e	7.2 d
	1.12	none	41.4 c	0.6 a
	2.24		28.4 ab	0.2 a
	0.0	4.48	74.8 e	7.1 d
	1.12	4.48	55.8 d	1.6 b
	2.24	4.48	27.5 a	0.2 a
	0.0	5.6	75.6 e	7.4 d
	1.12	5.6	59.3 d	2.9 c
	2.24	5.6	32.0 ab	0.2 a
	0.0	6.72	73.1 e	6.6 d
	1.12	6.72	57.2 d	2.8 c
	2.24	6.72	35.5 bc	0.3 a

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 4. Effects of NA and Carboxin on corn injury form buthidazole, 30 days after preemergence treatment[a].

	Buthidazole (kg/ha)	Antidotes	Rate (% w/w)	Shoot ht (cm/plant)	Shoot fresh wt (g/plant)
Main effects					
(i) Antidotes		none	0.0	54.7 ab	3.0 a
		NA	0.5	57.8 b	4.1 b
		Carboxin	0.5	53.7 a	2.8 a
(ii) Buthidazole	0.0			68.8 c	6.1 c
	0.56			61.9 b	4.0 b
	0.84			45.8 a	1.5 a
	1.12			45.1 a	1.5 a
Interactions	0.0	none	0.0	72.1 d	6.3 d
	0.56		0.0	63.0 c	4.3 c
	0.84		0.0	42.8 a	0.6 a
	1.12		0.0	41.0 a	0.7 a
	0.0	NA	0.5	61.0 c	5.5 d
	0.56		0.5	59.6 bc	4.3 c
	0.84		0.5	56.6 bc	3.4 bc
	1.12		0.5	54.0 b	3.1 b
	0.0	Carboxin	0.5	73.5 d	6.5 d
	0.56		0.5	63.0 c	3.4 bc
	0.84		0.5	37.9 a	0.6 a
	1.12		0.5	40.4 a	0.7 a

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 5. Effects of six herbicides on corn injury from buthidazole, 20 days after preemergence treatment[a].

	Buthidazole	Herbicides	Rate	Shoot ht	Shoot fresh wt
	(kg/ha)		(kg/ha)	(cm/plant)	(g/plant)
Main effects					
(i) Herbicides		none	0.0	31.2 b	1.6 a
		Alachlor	3.36	31.0 b	1.8 abc
		Metolachlor	3.36	32.1 bc	1.9 bc
		Diethatyl	3.36	31.1 b	2.0 c
		H-26910	3.36	31.7 bc	1.9 bc
		EPTC	3.36	28.6 a	1.7 ab
		Trifluralin	3.36	33.9 c	2.0 c
(ii) Buthidazole	0.0			37.5 c	3.1 c
	1.12			31.9 b	1.7 b
	2.24			24.7 a	0.8 a
Interactions	0.0			36.0 fg	2.7 d
	1.12	none		32.5 ef	1.6 c
	2.24			25.0 bc	0.7 a
	0.0	Alachlor	3.36	34.5 fg	2.6 d
	1.12		3.36	33.6 ef	1.9 c
	2.24		3.36	24.9 bc	0.9 ab
	0.0	Metolachlor	3.36	38.5 gh	3.2 e
	1.12		3.36	32.9 ef	1.8 c
	2.24		3.36	25.0 bc	0.7 a
	0.0	Diethatyl	3.36	35.1 fg	3.1 e
	1.12		3.36	30.2 de	1.7 c
	2.24		3.36	28.0 cd	1.1 b
	0.0	H-26910	3.36	40.0 hi	3.3 e
	1.12		3.36	32.3 ef	1.8 c
	2.24		3.36	22.8 ab	0.7 a

Table 5. (continued)

	Buthidazole	Herbicides	Rate	Shoot ht	Shoot fresh wt
	(kg/ha)		(kg/ha)	(cm/plant)	(g/plant)
Interactions	0.0	EPTC	3.36	35.5 fg	3.1 e
	1.12		3.36	29.8 de	1.5 c
	2.24		3.36	20.4 a	0.6 a
	0.0	Trifluralin	0.56	42.7 i	3.4 e
	1.12		0.56	32.3 ef	1.6 c
	2.24		0.56	26.6 bcd	0.9 ab

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 6. Effects of R-25788, Alachlor, and Diethatyl at 5.60 kg/ha on corn injury from buthidazole, 30 days after preemergence treatment[a].

	Buthidazole	Antidotes	Rate	Shoot ht	Shoot fresh wt
	(kg/ha)		(kg/ha)	(cm/plant)	(g/plant)
Main effects					
(i) Antidotes		none	0.0	47.0 b	2.6 ab
		R-25788	5.6	42.6 b	2.5 a
		Alachlor	5.6	43.7 b	3.1 b
		Diethatyl	5.6	36.0 a	2.5 a
(ii) Buthidazole	0.0			64.7 c	6.9 c
	1.12			36.3 b	1.0 b
	2.24			25.9 a	0.2 a
Interactions	0.0	none	0.0	73.2 e	7.2 e
	1.12		0.0	41.4 c	0.6 ab
	2.24		0.0	26.3 ab	0.2 ab
	0.0	R-25788	5.6	65.4 e	6.8 de
	1.12		5.6	33.4 bc	0.5 ab
	2.24		5.6	29.1 ab	0.1 a
	0.0	Alachlor	5.6	67.1 e	7.5 e
	1.12		5.6	40.7 c	1.5 c
	2.24		5.6	23.2 a	0.4 ab
	0.0	Diethatyl	5.6	53.2 d	6.1 d
	1.12		5.6	29.8 ab	1.1 bc
	2.24		5.6	24.8 ab	0.3 ab

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level according to Duncan's multiple range test.

Table 7. Effects of alachlor, metolachlor, and butylate + R-25788 at 2.24 kg/ha on corn injury from buthidazole, 30 days after preemergence treatment[a].

	Buthidazole	Herbicides	Rate	Shoot ht	Shoot fresh wt
	(kg/ha)		(kg/ha)	(cm/plant)	(g/plant)
Main effects					
(i) Herbicides		none	0.0	49.6 b	2.7 a
		alachlor	2.24	48.6 b	3.1 b
		metolachlor	2.24	43.4 a	2.6 a
		butylate + R-25788	2.24	49.9 b	2.6 a
(ii) Buthidazole	0.0			65.9 e	6.1 d
	0.42			61.4 d	4.6 c
	0.56			51.2 c	2.0 b
	1.12			34.5 b	0.3 a
	2.24			26.4 a	0.2 a
Interactions	0.0	none		67.9 g	6.5 h
	0.42			65.5 g	4.6 de
	0.56			51.4 f	2.0 b
	1.12			34.5 cd	0.3 a
	2.24			29.0 bc	0.2 a
	0.0	alachlor	2.24	65.1 g	6.2 gh
	0.42		2.24	64.1 g	5.1 ef
	0.56		2.24	54.9 f	3.4 c
	1.12		2.24	33.5 cd	0.5 a
	2.24		2.24	25.3 ab	0.2 a
	0.0	metolachlor	2.24	64.8 g	5.8 gh
	0.42		2.24	52.9 f	4.1 d
	0.56		2.24	43.6 e	2.5 b
	1.12		2.24	33.2 cd	0.3 a
	2.24		2.24	22.6 a	0.2 a
	0.0	butylate +	2.24	66.0 g	5.7 fg
	0.42	R-25788	2.24	63.2 g	4.6 de
	0.56		2.24	54.9 f	2.4 b
	1.12		2.24	36.9 d	0.3 a
	2.24		2.24	28.8 bc	0.2 a

[a] Means within columns for any given effect comparison followed by similar letters are not significantly different at the 5% level by Duncan's multiple range test.

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CHAPTER 9

Summary and Conclusions

Buthidazole was absorbed by both leaves and roots of alfalfa, quackgrass, corn, and redroot pigweed and moved acropetally following both foliar and root applications of ^{14}C-buthidazole. Redroot pigweed was the only plant species in which basipetal movement of ^{14}C-buthidazole was evident. However, rapid uptake by the roots and rapid movement to the shoots and leaves, via the xylem, appeared to be the main pathway of ^{14}C-buthidazole uptake and translocation in all four plant species. Differential absorption and translocation did not appear to be a factor contributing to buthidazole selectivity between alfalfa and quackgrass. There was no difference in buthidazole absorption between corn and redroot pigweed but the faster translocation of buthidazole from the roots to the shoots and leaves of redroot pigweed seedlings may play a role in the selective action of this herbicide between corn and redroot pigweed.

A differential rate of metabolism appeared to be the primary factor contributing to buthidazole selectivity both between alfalfa and quackgrass and between corn and redroot pigweed. A rapidly formed unidentified metabolite with Rf values ranging from 0.21 to 0.26 seems to be very important for the observed alfalfa and corn tolerance as its increase with time appeared coupled to a proportional decrease of the parent ^{14}C-buthidazole. Formation of this buthidazole derivative was very slow in redroot pigweed. Quackgrass formed only very small amounts of this metabolite at any time period. Other metabolites of buthidazole detected in the plant species examined, had Rf values corresponding to the dihydroxy, urea, methylurea, and amine derivatives of buthidazole. Further confirmation of the identity of these metabolites is needed prior to positive identification. Howevr, at present, this is technically difficult since the

obtained metabolites were present in small quantities, far below the resolution limitations of the existing analytical procedures.

Data obtained from the mode of action studies revealed that buthidazole and its analog, tebuthiuron, are very strong photosynthetic inhibitors, comparable to atrazine and diuron. The inhibitory effect was associated with the reducing side of photosystem II of the photosynthetic electron transport. A possible minor inhibition on the oxidizing side of photosystem II was also evident. Strong inhibition of total photosynthesis of all four plant species examined was also evident from *in vivo* measurements with an infrared CO_2 analyzer. However, corn photosynthetic rates were not affected by low rates of buthidazole applied preemergence. Postemergence application of buthidazole at rates as low as 0.28 kg/ha inhibited total photosynthesis of corn very rapidly. Inhibition of anthocyanin formation in corn did not appear to be connected to the effect of this herbicide on photosynthesis since low rates that inhibited anthocyanin biosynthesis did not inhibit total photosynthesis of corn following preemergence application of buthidazole. Reduction or prevention of starch accumulation in bundle sheath chloroplasts of corn plants treated with buthidazole and ultrastructural disruptions of some mesophyll chloroplasts of the same corn plants observed after postemergence application of buthidazole, further indicated that the herbicidal action of this compound is strongly associated with photosynthesis.

The transitory increase of corn and alfalfa respiration, observed only in plants treated with low buthidazole rates, may be indicative of the faster buthidazole metabolism in these tolerant species.

Buthidazole did not inhibit seed germination of all plant species examined in this research.

CDAA and NA were the most promising of six chemicals evaluated as antidotes to increase the tolerance of corn to higher rates of buthidazole. CDAA was more effective than NA, whereas R-25788 was totally ineffective. A ratio 1:3 (buthidazole:CDAA) appeared to be optimal for the protection effect. Even though this ratio (1:3) may not be practical, it indicates that buthidazole safety to corn can be enhanced chemically.

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