FACTORS INFLUENCING THE TOXICITY OF HEXAZINONE AND TEBUTHIURON IN SEVERAL WOODY SPECIES

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INTRODUCTION

Each of the three parts of this thesis is a separate manuscript to be submitted for publication in Weed Science, the journal of the Weed Science Society of America.

PART I

EFFECT OF TEBUTHIURON AND HEXAZINONE ON PHOTOSYNTHETIC CO_2 FIXATION IN SEVERAL WOODY SPECIES

EFFECT OF TEBUTHIURON AND HEXAZINONE ON PHOTOSYNTHETIC CO_2 FIXATION IN SEVERAL WOODY SPECIES

Abstract. The effect of tebuthiuron [N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea, hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] on photosynthetic CO_2 fixation was evaluated. Photosynthetic CO_2 fixation was significantly reduced in leaf-discs of winged elm (Ulmus alata Michx.), black walnut (Juglans nigra L.), bur oak (Quercus macrocarpa Michx.), and blackjack oak (Quercus marilandica Muech.) with all three herbicides following a 30 min incubation period. Hexazinone was the most effective inhibitor of photosynthetic $^{14}CO_2$ fixation with all four woody species. Concentrations of hexazinone, tebuthiuron, and diuron required to inhibit $^{14}CO_2$ fixation in winged elm leaf-discs by 50% (I_{50}) were 0.04, 6.27, and 3.13 µM respectively, whereas I_{50} values for black walnut were 4.40, 29.78, and 19.89 µM respectively. At concentrations of 10 µM and greater inhibition rates among the woody species was similar with a given herbicide.

Additional index words. Photosynthesis, leaf-discs, carbohydrates, ureas, triazines.

INTRODUCTION

Hexazinone and tebuthiuron are two recently introduced herbicides with excellent potential for the control of woody plants in rangelands. Injury symptoms following treatment with both herbicides are similar to those observed with known photosynthetic inhibiting herbicides such as simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] and fenuron [3-phenyl-1,1-dimethylurea] (1,6). Symptoms include foliar chlorosis followed by necrosis. Susceptible brush species usually defoliate several times during the first season and die either in the first or second season (11,14).

Numerous investigators have related the effects of the triazine (4,7,8,12) and urea (2,12) herbicides on photosynthesis. Means by which photosynthesis can be inhibited include interference: (a) with reproduction, development, structure, and integrety of chloroplasts; (b) with biosynthetic pathways that are involved in the production of output products; and (c) with photochemical induction pathways involved in the conversion of radiant energy to chemical energy (12).

Several s-triazine (3,5) and urea (13) herbicides have been shown to interfer with photosynthetic CO_2 fixation. Hatzios and Penner (9) reported that photosynthetic CO_2 fixation by enzymatically isolated leaf cells of navy bean (Phaseolus vulgaris L.) was very sensitive to tebuthiuron following a 30 min incubation period. Buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidione], a thiadiazol urea herbicide similar in structure to tebuthiuron, has been shown to inhibit whole plant photosynthesis measured as CO_2 uptake within 4 h following a postemergence application

(10).

Ashton et al. (3) demonstrated that simazine, trietazine [2-chloro-4-(diethylamino)-6-(ethylamino)-s-triazine], and simetone [2,4-bis(ethylamino)-6-methoxy-s-triazine] severely inhibited CO_2 fixation in red kidney beans (Phaseolus vulgaris L.) treated in the light. Couch and Davis (5) reported that atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] inhibited CO_2 fixation in corn (Zea mays L.), cotton (Gossypium hirsutum L.), and soybeans (Glycine max L.).

The objective of this study was to evaluate the effects of tebuthiuron and hexazinone on $^{14}CO_2$ fixation in bur oak, blackjack oak, black walnut, and winged elm.

METHODS AND MATERIALS

$^{14}CO_2$ fixation. Fully expanded leaves of bur oak, blackjack oak, black walnut, and winged elm were harvested in the field, washed in cold tap water, blotted dry, and 9 mm leaf-discs removed with a sharp cork borer. Approximately 55 leaf-discs were vacuum infiltrated with varying concentrations of herbicide solution for 30 min at room temperature after which 5 leaf-discs were randomly selected and placed on filter paper. The filter paper was positioned in the upper one-half of a modified Warburg flask containing 0.625 µCi of aqueous $Na_2{}^{14}CO_3$ (sp. act. 50 mCi/mM) in the bottom of the flask and 10% lactic acid in the side arm. Air was passed over a soda lime absorber and drawn through two 4N KOH traps and the reaction vessel for 5 min to remove endogenous CO_2. The reaction vessel was sealed and tilted to allow the 10% lactic acid to react with the $Na_2{}^{14}CO_3$ thereby generating labeled carbon dioxide. Sixty seconds were allowed for the reaction to proceed to completion

generating approximately 400 ppmv CO_2. The reaction vessel was submersed in 4 cm of water and illuminated for 3 min with a 500-w reflectorized photo flood lamp. Light intensity was 63,000 lux (1700 µE $m^{-2} sec^{-1}$) at the water surface. Following the 3 min assimilation period, the remaining $^{14}CO_2$ was evacuated under vacuum through a soda lime absorber and two 4N KOH traps for 5 min. Each leaf-disc was placed in a liquid scintillation vial containing 15 ml of $^{14}CO_2$UNTSORB counting cocktail. The vials were left standing overnight prior to counting in a liquid scintillation spectrometer. Four runs of 5 leaf-discs were assayed for each treatment. Data presented are the percent inhibition means of the four runs (20 leaf-discs) in a completely randomized factorial design. The data were subjected to an analysis of variance and the differences tested for significance at the 5% probability level. I_{50} values (concentration of herbicide necessary to inhibit $^{14}CO_2$ fixation by 50%) were predicted using linear regression analysis of percent inhibition against herbicide concentration for each of the woody species.

Herbicide stock solution. Herbicide stock solutions of hexazinone, tebuthiuron, and diuron were prepared in acetone at concentration intervals ranging from 0.01 to 1000 µM. Analytical grade hexazinone (99^+% pure) and technical grades tebuthiuron (96.5% pure) and diuron (98.6% pure) were used. Herbicide stock solutions were stored at -5 C. The controls received an equivalent amount of acetone (3%, v/v) in all assays.

RESULTS

Rates of photosynthetic $^{14}CO_2$ fixation by leaf-discs from all four

woody species were significantly reduced by all three herbicides (Tables 1,2,3). Hexazinone inhibited photosynthetic $^{14}CO_2$ fixation in winged elm leaf-discs to a greater extent than in the other three species at all concentrations evaluated (Table 1). Inhibition of $^{14}CO_2$ fixation in winged elm leaf-discs ranged from 44% at the 0.01 µM concentration to a maximum of 87% at the 1000 µM concentration. This compares to an inhibition extreme of 10 and 70% for black walnut. At hexazinone concentrations of 0.01 and 0.10 µM, photosynthetic CO_2 fixation in leaf-discs of bur oak was inhibited more than in black walnut or blackjack oak. However, no significant differences in percent inhibition were observed among black walnut, bur oak, or blackjack oak at concentrations at or above 10 µM. I_{50} values for hexazinone ranged from 0.04 µM for winged elm to 4.40 µM for black walnut. I_{50} values for bur oak and blackjack oak were intermediate being 1.02 and 2.63 µM respectively.

Tebuthiuron was more inhibitory of $^{14}CO_2$ fixation in leaf-discs of winged elm than in the other three species (Table 2). The calculated I_{50} value for winged elm was 6.27 µM compared to I_{50} values of 15.09, 17.36, and 29.78 µM respectively for blackjack oak, bur oak, and black walnut. At concentrations of 10 µM or greater no significant differences were found in percent inhibition among the four woody species. Inhibition of $^{14}CO_2$ fixation ranged from a low of 11% in black walnut at the 0.01 µM concentration to a maximum of 70% in bur oak at the 1000 µM concentration.

At the 0.1 and 1.0 µM concentrations, diuron was more inhibitory to photosynthetic $^{14}CO_2$ fixation in winged elm than in black walnut (Table 3). At higher concentrations percent inhibition tended to level out and as a result differences between the two woody species were not as great.

Percent inhibition of $^{14}CO_2$ fixation by diuron ranged from 8% in black walnut at the lowest concentration to 65% in winged elm at the highest concentration. I_{50} values following treatment with diuron (3.13 and 19.89 µM for winged elm and black walnut respectively) were comparable to those obtained with tebuthiuron, both of which were at least 5 times larger than the I_{50} values obtained with hexazinone.

DISCUSSION

Photosynthetic fixation of carbon dioxide was reduced in all four woody species with all three herbicides. Carbon dioxide fixation in winged elm was more sensitive to a given herbicide than it was in the other three species. In general no differences in percent inhibition were observed among the species treated with a given herbicide at or above 10 µM. Hexazinone was the most efficient inhibitor of photosynthetic carbon dioxide fixation in all four woody species based on calculated I_{50} values. Inhibition rates obtained with tebuthiuron are similar to those reported by Hatzios and Penner (9) for tebuthiuron inhibition of isolated leaf cells of navy bean at concentrations of 1.0 µM and below. However, the amount of inhibition continued to increase with the higher concentrations whereas at the higher concentrations of this study percent inhibition tended to level off more rapidly. The large I_{50} value calculated for black walnut treated with tebuthiuron is consistent with data demonstrating black walnut's resistance to tebuthiuron under field conditions. Also, the difference in I_{50} values between winged elm's ability to fix $^{14}CO_2$ and that of blackjack oak would explain why tebuthiuron injury to winged elm was detected earlier under field conditions than injury to blackjack oak (14). Differences in the ability

of a given tree species to absorb, transport, or metabolize a given herbicide may account for the differing abilities among the woody species to tolerate a given herbicide.

The results obtained from this study demonstrate that both tebuthiuron and hexazinone are efficient inhibitors of photosynthetic carbon dioxide fixation. However, death results too rapidly to be explained by starvation, therefore secondary effects resulting from electron transport inhibition probably explain the cause of death (15). Inhibition of electron transport would explain the rapid inhibition of photosynthetic carbon dioxide fixation in this and other studies (9,10).

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Table 1. Percent inhibition of $^{14}CO_2$ fixation in leaf-discs of four woody species following treatment with hexazinone.[a]

Hexazinone Concentration	Winged Elm	Bur Oak	Blackjack Oak	Black Walnut
(µM)	——————(% Inhibition)——————			
0.01	44 hi	23 j	11 k	10 k
0.10	64 def	45 h	28 j	34 ij
1	76 bc	56 fg	47 gh	43 hi
10	81 ab	59 def	57 ef	56 fg
100	81 ab	62 def	68 cde	63 def
1000	87 a	66 cdef	68 cde	70 cd
I_{50}	0.04 µM	1.02 µM	2.63 µM	4.40 µM

[a]Values followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at the 5% probability level.

Table 2. Percent inhibition of $^{14}CO_2$ fixation in leaf-discs of four woody species following treatment with tebuthiuron.[a]

Tebuthiuron Concentration	Winged Elm	Bur Oak	Blackjack Oak	Black Walnut
(µM)	(% Inhibition)			
0.01	21 no	25 mn	18 o	11 p
0.10	36 kl	28 m	27 m	23 mno
1	50 gh	44 ij	40 jk	34 l
10	54 fg	56 ef	50 gh	47 hi
100	59 def	61 bcd	61 bcd	60 cde
1000	68 a	70 a	66 ab	65 abc
I_{50}	6.27 µM	17.36 µM	15.09 µM	29.78 µM

[a]Values followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at the 5% probability level.

Table 3. Percent inhibition of $^{14}CO_2$ fixation in leaf-discs of two woody species following treatment with diuron.[a]

Diuron Concentration	Winged Elm	Black Walnut
(µM)	(% Inhibition)	
0.01	13 h	8 h
0.10	32 f	25 g
1	47 e	37 f
10	55 bcd	47 de
100	60 bc	55 bcd
1000	65 a	62 ab
I_{50}	3.13 µM	19.89 µM

[a]Values followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at the 5% probability level.

PART II

INFLUENCE OF TEBUTHIURON AND HEXAZINONE ON ELECTRON TRANSPORT AND OXYGEN EVOLUTION IN SPINACH AND SEVERAL WOODY PLANTS

INFLUENCE OF TEBUTHIURON AND HEXAZINONE ON ELECTRON TRANSPORT AND OXYGEN EVOLUTION IN SPINACH AND SEVERAL WOODY SPECIES

Abstract. The effects of tebuthiuron [N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea], hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] on noncyclic basal electron transport (Hill reaction) and oxygen evolution was evaluated. Chloroplasts used in the electron transport assays were isolated from black walnut (Juglans nigra L.), blackjack oak (Quercus marilandica Muech.), post oak (Quercus stellata Wangenh.), winged elm (Ulmus alata Michx.), and spinach (Spinacia oleracea L.). Photochemical activity was measured spectrophotometrically with ferricyanide as the electron acceptor. Concentrations of tebuthiuron and hexazinone required to inhibit the Hill reaction in spinach chloroplasts by 50% (I_{50}) were 0.72 and 1.10 µM respectively. None of the herbicides tested were able to inhibit electron transport in the isolated tree chloroplasts by 50%. This was attributed to the presence of polyethelene glycol which had to be used in the extraction of the tree chloroplasts to protect them from phenols present in the foliage. Short term oxygen evolution was inhibited by all three compounds in leaf-discs of winged elm and black walnut. Hexazinone was more effective than tebuthiuron in reducing oxygen evolution from winged elm leaf-discs, whereas tebuthiuron was

more effective in inhibiting oxygen evolution from leaf-discs of black walnut.

Additional index words. Photosynthesis, Hill reaction, isolated chloroplasts, ureas, triazines.

INTRODUCTION

The photosynthetic process is based on a light-induced electron transfer from water to $NADP^+$ which results in the formation of oxygen. Artificial electron acceptors can be substituted for $NADP^+$, which give rise to oxygen evolution but involve only a short segment of the oxidation chain (14). Artificial electron acceptors include ferricyanide (FeCN) and 2,6-dichlorophenolindophenol (DCPIP) which accept electrons from the electron chain intermediates (3). Reduction of the electron acceptor can be measured spectrophotometrically.

A number of investigators have related the effects of the urea and triazine herbicides on photosynthesis (3,11,13,19,20). Most of the research places the most sensitive site of action for these families on photosystem II and oxygen evolution. Bishop (2) observed that diuron inhibited the mechanism involved in oxygen evolution in Scenedesmus. Zweig et al. (22) reported that diuron inhibited oxygen evolution in Chlorella pyrenoidosa Chick. almost immediately after addition. Photosynthetic oxygen evolution was reduced 58% in cotton (Gossypium hirsutum L.) and 46% in cucumber (Cucumis sativus L.) leaf tissue after a 90 min treatment with 4.3×10^{-5} M fluometuron [1,1-dimethyl-3-(∝,∞,∞-trifluoro-m-tolyl)urea] (16).

Cooke (5) and others (2,7,13,20) have established that the ureas are potent inhibitors of the Hill reaction in photosynthesis. Levine

(10) reported that diuron prevented the acceptance of electrons by cytochromes in photosystem II. The triazine herbicides apparently act at the same site as the phenylureas (14). These compounds inhibit basal electron transport, methylamine-uncoupled electron transport, and noncyclic electron transport.

Hatzios and Penner (8) found that tebuthiuron inhibited uncoupled electron transport very strongly in isolated spinach chloroplasts. They concluded that the site of electron transport inhibition is at the reducing side of photosystem II. York et al. (21) reached similar conclusions working with buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidione].

The objectives of this research were to (1) evaluate the effects of tebuthiuron, hexazinone, and diuron on noncyclic basal electron transport, and (2) to determine the effects of these compounds on oxygen evolution by winged elm and black walnut leaf-discs.

METHODS AND MATERIALS

Electron transport. Winged elm, post oak, blackjack oak, black walnut, and commercially purchased spinach were the sources of chloroplasts used in the noncyclic basal electron transport studies.

Tree chloroplasts were isolated by cutting 50 g of fresh leaf tissue into 1 cm segments and vacuum infiltrating with 100 ml of extraction medium for 30 min. The extraction media contained 400 mM sucrose, 50 mM tris-HCL buffer (pH 8.0), 10 mM NaCl, and 20 mM ascorbate. No activity was found in the isolated tree chloroplasts until a protectant [polyethelene glycol 3000 (3%, w/v)] was added to the extraction media. The leaf segments were ground with a mechanical blender and the

extract filtered through four layers of cheesecloth. The filtered homoginate was then centrifuged at 500 x g for 2 min and the precipitate discarded. The chloroplasts were collected by centrifugation of the supernatant at 1000 x g for 5 min. The supernatant was discarded and the chloroplast pellet was resuspended in 25 ml of pickup medium containing 400 mM sucrose, 50 mM tris-HCL buffer (pH 8.0), and 10 mM NaCl. The suspension was centrifuged at 1000 x g for 5 min and the supernatant discarded. The chloroplasts were resuspended in 4 ml of pickup medium and chlorophyll concentration determined by the method of Arnon (1). All steps in the isolation process were conducted at 4 C.

Spinach chloroplasts were isolated in a manner similar to the tree chloroplasts with two exceptions. The extraction medium did not contain polyethelene glycol 3000 nor was the foliage vacuum infiltrated with extraction media prior to grinding.

Chloroplast stock suspensions were diluted with pickup medium to provide a concentration of 0.15 mg chlorophyll/ml, and 0.5 ml of the chloroplast suspension was added to 0.5 ml 0.1 M NaCl, 0.25 ml of 0.2 M tris-HCL (pH 8.0), 1.0 ml 2 mM $K_3Fe(CN)_6$, 2.70 ml distilled water, and 0.05 ml of inhibitor solution to make 5.0 ml total volume.

Several investigators (8,21) have observed that electron transport inhibition by the thiadiazol urea herbicides is time-dependent. Preliminary experiments were performed with chloroplasts preincubated with 1 µM concentrations of tebuthiuron or hexazinone at time intervals ranging from 0 to 10 min before being exposed to light for the electron transport measurements. Optimum inhibition of electron transport occurred with the 5 min preincubation of herbicide plus chloroplasts; therefore, this procedure was utilized in the electron transport assays.

Reactions were performed in test tubes illuminated from the side by 500-w reflectorized photo flood lamps for 3 min. A water filter of $CuSO_4$ (2%, w/v) was interposed between the light source and the reaction tubes to trap heat emitted by the lamps. Light intensity was 35,000 lux (950 $\mu E\ m^{-2} sec^{-1}$). Temperature of the reaction mixture during illumination was 26 C. Reactions were terminated by the addition of 1.0 ml of 10% trichloroacetic acid, and the precipitated protein was removed by centrifugation at 2000 x g for 10 min. Ferricyanide reduction in the supernatants was measured as a decrease in absorbance at 420 nm. Determinations were made in triplicate with two separate chloroplast extractions for black walnut, post oak, and blackjack oak, three extractions for spinach, and four extractions for winged elm. Data presented are the percent inhibition means of each treatment in a completely randomized factorial design. The data were subjected to an analysis of variance and Duncan's New Multiple Range Test was used to test for significant differences at the 5% probability level. I_{50} values (concentration of herbicide necessary to inhibit electron transport 50%) were predicted using linear regression analysis of percent inhibition against herbicide concentration (0.01, 0.10, and 1.0 μM) for each compound where applicable.

Oxygen evolution. Polarographic measurements of oxygen evolution were conducted in a double-walled reaction vessel using a YSI 53 oxygen monitor adapted with a YSI Clark electrode. A combination water bath and circulator regulated the temperature of the reaction vessel at 32 C. Forty winged elm or black walnut leaf-discs (5 mm in diameter) were placed in the reaction vessel containing 5 ml of distilled water saturated with oxygen. Respiration was allowed to occur in the dark

until the solution reached approximately 55% saturation. The samples were illuminated with a light intensity of approximately 60,000 lux. Fifty microliters of herbicide solution was introduced into the vessel after a constant rate of oxygen evolution was established and the effect on oxygen evolution monitored for 15 min. Changes in oxygen concentration were recorded by a strip chart recorder. Data presented are the means of three replications in a split plot design. The data were subjected to an analysis of variance and the differences tested for significance at the 5% probability level.

Herbicide stock solution. Herbicide stock solutions of hexazinone, tebuthiuron, and diuron were prepared in acetone at concentration intervals ranging from 0.01 to 1000 µM. Analytical grade hexazinone (99^{+}% pure) and technical grades tebuthiuron (96.5% pure) and diuron (98.6% pure) were used. Herbicide stock solutions were stored at -5 C. The control received an equal amount of acetone (3%, v/v) in all assays.

RESULTS AND DISCUSSION

Electron transport. The effects of tebuthiuron, hexazinone, and diuron on noncyclic basal electron transport (Hill reaction) in chloroplasts isolated from the various woody species are shown in Tables 1 and 2. Percent inhibition with a given herbicide was not consistantly rate responsive in chloroplasts isolated from black walnut, post oak, and blackjack oak (Table 1). There was a rate response in chloroplasts isolated from winged elm with both diuron and tebuthiuron (Table 2). Diuron at 100 µM reduced electron transport 21% while the same concentration of tebuthiuron resulted in 15% inhibition.

Results obtained from these studies were not conclusive. There was

some inhibition of electron transport in chloroplasts isolated from post oak with all concentrations of tebuthiuron and with the two higher concentrations of hexazinone. In addition, chloroplasts isolated from black walnut were inhibited about 17% at all concentrations of hexazinone. None of the herbicides inhibited electron transport more than 25%. The inability of these compounds to more effectively inhibit the electron transport process could possibly be due to the presence of polyethelene glycol (PEG). PEG was added to the extraction medium to prevent chloroplast inactivation from inhibitors present in the tree foliage during the extraction process (18). Tree chloroplasts prepared in the absence of PEG (9,15), and without vacuum infiltration (9) did not exhibit activity. It is presumed that the beneficial effect of PEG occurs through the binding of phenols and the stabilization of chloroplast structure (4). Based on the results obtained in these studies, it is probable that PEG prevented the ready access of the herbicides to the chloroplast membranes.

Electron transport in spinach chloroplasts was effectively inhibited by tebuthiuron, hexazinone, and diuron (Table 3). All three compounds at concentrations of 1.0 µM and higher were strong inhibitors of electron transport. The inhibition levels obtained with hexazinone, tebuthiuron, and diuron at 1.0 µM, were 48, 69, and 92% respectively. Diuron was more inhibitory than tebuthiuron and hexazinone at all concentrations used in the study. Hexazinone was at least twice as effective as tebuthiuron at the lower concentrations of 0.01 and 0.10 µM. However, at concentrations of 1.0 µM and above, tebuthiuron significantly inhibited electron transport more than hexazinone. The calculated I_{50} values for diuron, tebuthiuron, and hexazinone were 0.30,

0.72, and 1.10 µM respectively. These I_{50} values are within the range of I_{50} values reported for electron transport inhibition for several substituted urea and s-triazine herbicides (3,12,13)

Oxygen evolution. The results of short term exposure of tebuthiuron, hexazinone, and diuron on oxygen evolution from leaf-discs of winged elm and black walnut are shown in Table 4. No significant herbicide by rate interactions occurred with either tree species, therefore only main effects will be discussed. Diuron and hexazinone (averaged over herbicide concentration) were more effective in reducing oxygen evolution from winged elm leaf-discs than tebuthiuron. Oxygen evolution was inhibited 25.6 and 25% with diuron and hexazinone respectively while tebuthiuron reduced oxygen evolution 17.6%. Inhibition of oxygen evolution (averaged over herbicide) was rate responsive. Inhibition ranged from 9.6% at the 0.01 µM concentration to 42.3% at the 100 µM concentration.

Diuron and tebuthiuron (averaged over concentration) were more effective than hexazinone in reducing oxygen evolution in black walnut leaf-discs. Diuron and tebuthiuron inhibited oxygen evolution 36.0 and 31.3% respectively, whereas hexazinone reduced oxygen evolution only 19%. The 100 µM concentration significantly reduced oxygen evolution by black walnut leaf-discs more than the 0.01 or 1 µM concentrations.

Both tebuthiuron and hexazinone inhibited oxygen evolution. It is possible that greater inhibition of oxygen evolution could have resulted if exposure had been longer than 15 min. Rogers and Funderburk (16) observed a positive correlation in percent inhibition of oxygen evolution with time. Percent inhibition of fluometuron treated cotton leaf sections ranged from 11% after 15 min to 58% after a 90 min exposure.

Results obtained from the oxygen evolution studies coupled with the data demonstrating electron transport inhibition in isolated spinach chloroplasts support previous assumptions suggesting that tebuthiuron (8) and hexazinone (17) were photosynthetic inhibitors. These results demonstrate that both tebuthiuron and hexazinone are effective inhibitors of photosystem II and suggest that this is a major mechanism of action for both herbicides. Electron transport inhibition would explain the rapid inhibition of CO_2 fixation in the previous study. Inhibition of electron transport would result in a deficiency of ATP and NADPH necessary for CO_2 fixation (21). Complete blockage of electron transport would conceivably block all CO_2 fixation, provided that this was the only energy source and CO_2 fixation pathway (6). Since 100 µM concentrations of hexazinone, tebuthiuron, and diuron were found to inhibit electron transport 88, 97, and 99% respectively, it is possible that insufficient levels of herbicide reached the site of action to cause a complete blockage of electron transport allowing CO_2 fixation to occur.

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Table 1. Percent inhibition of electron transport (Hill reaction) in chloroplasts isolated from black walnut, post oak, and blackjack oak by tebuthiuron, hexazinone, and diuron.[a]

Concentration	Black Walnut	Post Oak	Blackjack Oak
(μM)	(% Inhibition)[b]		
Tebuthiuron			
0.1	2	25	3
1.0	5	17	8
10.0	6	19	16
Hexazinone			
0.1	17	7	4
1.0	17	22	7
10.0	18	18	4
Diuron			
0.1	5	0	7
1.0	1	1	13
10.0	4	8	14

[a]No significant differences were observed.

[b]Control values averaged 148, 273, and 228 μmoles of ferricyanide reduced/mg chlorophyll/h for black walnut, post oak, and blackjack oak respectively.

Table 2. Percent inhibition of electron transport (Hill reaction) in chloroplasts isolated from winged elm by tebuthiuron, hexazinone, and diuron.[a]

Concentration	Tebuthiuron	Hexazinone	Diuron
(μM)	(% Inhibition)[b]		
0.1	4 d	3 d	4 d
1.0	3 d	4 d	9 bcd
10.0	7 cd	8 cd	13 bc
100.0	15 ab	9 bcd	21 a

[a]Values followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at the 5% probability level.

[b]Control values averaged 237 μmoles of ferricyanide reduced/mg chlorophyll/h.

Table 3. Percent inhibition of electron transport (Hill reaction) in chloroplasts isolated from spinach by tebuthiuron, hexazinone, and diuron.[a]

Concentration	Tebuthiuron	Hexazinone	Diuron
(μM)	(% Inhibition)[b]		
0.01	3 l	11 k	19 j
0.1	11 k	24 i	50 h
1.0	69 g	48 h	92 cd
10.0	90 de	75 f	98 ab
100.0	97 abc	88 e	99 a
I_{50}	0.72 μM	1.10 μM	0.30 μM

[a]Values followed by the same letter are not significantly different according to Duncan's New Multiple Range Test at the 5% probability level.

[b]Control values averaged 282 μmoles of ferricyanide reduced/mg chlorophyll/h.

Table 4. Percent inhibition of oxygen evolution in leaf-discs of winged elm and black walnut by tebuthiuron, hexazinone, and diuron.[a]

Concentration	Tebuthiuron	Hexazinone	Diuron	Average
(μM)		(% Inhibition)		
Winged elm				
0.01	10	7	12	9.6 c
1.0	12	19	18	16.3 b
100.0	31	51	45	42.3 a
Average	17.6 b	25.6 a	25.0 a	
Black walnut[b]				
0.01	24	7	27	19.3 b
1.0	26	13	28	22.3 b
100.0	44	37	53	44.6 a
Average	31.3 a	19.0 b	36.0 a	

[a]Main effect averages followed by the same letter are not significantly different at the 5% probability level.

[b]Hexazinone treatments were replicated twice at the 0.01 and 1.0 μM concentrations.

PART III

ABSORPTION, TRANSLOCATION, AND DEGRADATION OF TEBUTHIURON AND HEXAZINONE IN SEVERAL WOODY SPECIES

ABSORPTION, TRANSLOCATION, AND DEGRADATION OF TEBUTHIURON AND HEXAZINONE IN SEVERAL WOODY SPECIES

Abstract. Four-month-old seedlings of winged elm (Ulmus alata Michx.) and 12-to-15 month old seedlings of bur oak (Quercus macrocarpa Michx.), black walnut (Juglans nigra L.), eastern redcedar (Juniperus virginiana L.), and loblolly pine (Pinus taeda L.) were treated in nutrient solution with ring-labeled ^{14}C-tebuthiuron [N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea] or ^{14}C-hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione]. ^{14}C was detected in all sections of winged elm at the 4 h harvest following treatment with ^{14}C-tebuthiuon and ^{14}C-hexazinone indicating rapid uptake and distribution of both compounds from the nutrient culture. Root absorption of the tebuthiuron label by the older species occurred in the order: loblolly pine > bur oak > black walnut = eastern redcedar. The sequence of ^{14}C-hexazinone absorbed by the roots was:loblolly pine > black walnut ≥ bur oak = eastern redcedar. Foliar accumulation of the tebuthiuron label occurred in the order: bur oak (S) > loblolly pine (I) > eastern redcedar (R) = black walnut (R). Activity in the foliage following treatment with labeled hexazinone occurred in the order: loblolly pine (R) > bur oak (S) ≥ black walnut (S) = eastern redcedar (I). Two metabolites of tebuthiuron were identified in eastern redcedar and one metabolite was identified in bur oak and loblolly pine. Three

metabolites of hexazinone were isolated from loblolly pine, one from the roots and two from the foliage. The presence of the three metabolites suggests that loblolly pine is resistant to hexazinone as a result of its ability to degrade hexazinone rather than its ability to limit uptake.

Additional index words. Quercus macrocarpa, Juglans nigra, Juniperus virginiana, Pinus taeda, Ulmus alata.

INTRODUCTION

Tebuthiuron and hexazinone are both used for the control of woody plants. Although the structures of the compounds are different, their main herbicidal mode of action involves interference with photosynthesis (8,13). Several reports have related the response of plants to substituted urea and triazine herbicides to their uptake and distribution patterns (16,17,18). Steinert and Stritzke (20) reported that the uptake and translocation of ^{14}C-tebuthiuron was greater in common ragweed (Ambrosia artemisiifoiia L.) than in the less sensitive rye (Secale cereale L. 'Elbon'). Other investigators have reported that differences in root absorption (6) and translocation (7,9) were not factors contributing to the selectivity of thiadiazol urea herbicides. These investigators concluded that differential rates and types of metabolism contributed to selectivity among the species evaluated. Martin and Morton (11) observed a positive correlation between the breakdown of tebuthiuron and plant resistance. Hatzios and Penner (7) concluded that the rate of metabolism contributed to buthidazole [3-5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidione] selectivity between alflafa (resistant) and quackgrass

(susceptible). Shimabukuro (15) found both quantitative and qualitative differences in atrazine [2-chloro-4(ethylamino)-6-(isopropylamino)-s-triazine] metabolism between resistant and susceptible plant species. Thompson (21) showed that several species and varieties of Setaria and Panicum degraded atrazine and propazine [2-chloro-4,6-bis(isopropylamino)-s-triazine] mainly by peptide conjugation, and the degree of conjugation was related to the degree of tolerance to these herbicides.

The purpose of this study was to determine the role of absorption, translocation, and degradation in the selectivity of tebuthiuron and hexazinone in several woody species.

METHODS AND MATERIALS

The woody species selected for this investigation were chosen on the basis of their response to tebuthiuron and hexazinone under field conditions. Winged elm and bur oak[1,2] are susceptible to tebuthiuron and hexazinone. Loblolly pine is resistant to hexazinone (4) and intermediate to tebuthiuron (12). Black walnut and eastern redcedar are resistant to tebuthiuron but are susceptible and intermediate to hexazinone respectively.[3]

Uptake and distribution. Winged elm seeds were collected near Seminole, Oklahoma, in April of 1981. Seeds were germinated in an organic compost and grown for 4 months in a growth chamber under the

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[1]Personal Communication, Benny Eaton, Research Scientist, Lilly Research Laboratories.

[2]Personal Communication, Larry Shelton, Research Scientist, E.I. duPont deNemours and Co. Inc.

[3]Stritzke, J.F., unpublished data.

following conditions: photoperiod of 14 h, day temperature of 31 ± 2 C, night temperature of 25 ± 2 C, and a light intensity of 18 klux. Three days prior to treatment, the winged elm seedlings were removed from the compost, sorted for uniformity, and placed in 75 ml green bottles containing well aerated half-strength Hoagland's solution. Iron was supplied at a concentration 1½ times that of the original recipe. The pH of the solution was adjusted to 5.9 using KOH to simulate typical soil pH conditions encountered in the field. The seedlings were transferred to fresh nutrient solution containing 10 µl of ^{14}C-tebuthiuron (0.298 µCi of thiadiazol ring labeled; sp. act. 17.4 µCi/mg) or ^{14}C-hexazinone (0.135 µCi of ring labeled; sp. act. 7.11 µCi/mg) in ethanol. This gave a concentration in the nutrient solution of 1 µM of labeled herbicide. The experiment was arranged in a stripped factorial design with two time intervals and two herbicides replicated eight times. The experiment was repeated and the data pooled. Herbicide concentrations are expressed as nanamoles per gram (nmoles/g) dry weight of tissue. The winged elm seedlings were harvested 4 and 24 h after treatment. The seedlings were divided into roots, lower stem, upper stem, and individual leaves. The sectioned plant parts were then freeze dried, weighed, and combusted in a Harvey Biological Material Oxidezer for 4 min. The resulting $^{14}CO_2$ was trapped in 15 ml of $^{14}CO_2$UNTSORB counting cocktail and quantified in disintegrations per minute (DPM) using a liquid scintillation spectrometer.

Seedlings of bur oak, black walnut, eastern redcedar, and loblolly pine were obtained from the Oklahoma State Nursery located near Washington, Oklahoma, in February of 1980. The 12-to-15 month old seedlings were planted in an organic compost fortified with nutrients

and grown in the greenhouse until needed. Four days prior to treatment these older seedlings were placed in 500 ml amber glass bottles containing 450 ml of aerated Hoagland's solution. At treatment the seedlings were transferred to fresh solution containing 30 µl of ^{14}C-tebuthiuron (0.898 µCi) or ^{14}C-hexazinone (0.40 µCi) in ethanol. This gave a concentration of 0.5 µM of labeled herbicide. All four species were harvested 4 and 24 h after treatment. In addition, eastern redcedar and bur oak were harvested 72 h after treatment. The deciduous seedlings were sectioned into roots, lower stem, upper stem, and individual leaves. The coniferous species were sectioned into roots and foliage. Foliage included both needles and small twigs randomly selected from either the lower or upper half of the foliage of eastern redcedar while only needles were randomly selected from loblolly pine. Each of the sectioned plant parts were frozen and lypholized. Subsamples (approximately 250 mg) of each plant part were oxidized and the resulting $^{14}CO_2$ trapped as previously described for winged elm.

Experiments with the older seedlings were conducted in a growth chamber under the following experimental conditions: photoperiod of 14 h, day temperatures of 33 ± 2 C, night temperatures of 25 ± 2 C, and a light intensity of 31 klux. Experiments were arranaged in a stripped factorial design with four tree species, two or three time intervals, and two herbicides. Four to seven replications of each combination of the main effects was used. Each experiment was repeated and the data pooled. This resulted in a total of 8, 11, 11, and 13 replications for bur oak, eastern redcedar, loblolly pine, and black walnut respectively. The data were subjected to an analysis of variance and the differences tested for significance at the 5% probability level.

Degradation. Tissue remaining from the experiments involving the older seedlings were used to determine the amount of tebuthiuron and hexazinone degradation. Residues from the replicates of each species were combined for each herbicide treatment keeping leaves and roots separate. Remaining individual leaves of the deciduous species were pooled into two groups, lower and upper foliage, for the extraction procedure. Pooled tissue groups were homogingized in 200 ml of methanol and vacuum filtrated. The residue was refluxed for 1 h with a 1:1 ratio of methanol and H_2O. The two extracts were combined and the methanol evaporated off by rotary vacuum evaporation at 50 C. The volume of the remaining aqueous phase was determined and sodium chloride added to make a 5% NaCl solution. This fraction was partitioned with an equal volume of cyclohexane to remove the remaining chlorophyll (three to five washes) and to obtain the parent compound. Nonconjugated metabolites were extracted from the aqueous phase by partitioning with three washes of dichloromethane. The remaining aqueous phase was hydrolized for 1 h with a 1:1 ratio of 3N HCL. Equal volumes of ethyl acetate were used to wash the aqueous fraction. The three fractions (cyclohexane, dichloromethane, and ethyl acetate) were evaporated to near dryness and quantitatively brought back to 10 ml.

For tebuthiuron degradation, 2 µl aliquots of the tebuthiuron treated samples were spotted on silica gel plates. Chromatograms were developed in hexane:acetone (60:40, v/v). The plates were air dried and viewed under ultraviolet light after the solvent front had moved 15 cm to determine the Rf values of the reference standards and the unknowns.

Ten µl aliquots of the hexazinone treated samples were spotted on

the silica gel plates and the chromatograms allowed to develop in the same manner. The chromatograms were developed in ethyl acetate: methanol (9:1, v/v). Rf values of ^{14}C-hexazinone and its degradation products were estimated by assaying 0.5 cm sections scraped from the region between the origin and the solvent front for radioactivity.

RESULTS AND DISCUSSION

Uptake and distribution. Concentrations of ^{14}C found in the roots, stems, and foliage of four-month-old winged elm seedlings following treatment with labeled tebuthiuron or hexazinone are shown in Tables 1 and 2. Both compounds were readily taken up by the winged elm seedlings as ^{14}C was detected in the foliage at the 4 h harvest. No significant differences in activity was detected between tebuthiuron and hexazinone at a given plant part and harvest time. The amount of activity in the roots did not increase appreciably from the 4 to 24 h treatment time with either herbicide (Table 1). The concentration of ^{14}C in both the upper and lower stems of winged elm increased with time when treated with ^{14}C-tebuthiuron, whereas with the ^{14}C-hexazinone treatment, the level of activity increased only in the lower stem. The activity of ^{14}C in the foliage of winged elm indicates that the initial accumulation of ^{14}C (4 h) was similar in leaves of all ages for a given herbicide (Table 2). However, by 24 h, the older bottom leaves contained significantly more labeled activity. This would infer that the movement of both tebuthiuron and hexazinone was via the transpiration stream as is typical of apoplastic movement. Similar conclusions were reached by other investigators (5,6,7) evaluating the absorption and translocation of buthidazole in soybeans (Glycine max L.), redroot pigweed

(Amaranthus retroflexis L.), and alfalfa (Medicago sativa L.) respectively.

The concentration of ^{14}C found in the roots, stems, and foliage of black walnut, bur oak, eastern redcedar, and loblolly pine after treatment with labeled tebuthiuron or hexazinone are shown in Tables 3, 4, and 5. The major site of radioactive accumulation for all species was in the roots as 58 to 96% of the absorbed tebuthiuron label and 50 to 83% of the hexazinone label was recovered in the roots of the older trees at the 24 h harvest (Table 3). Loblolly pine accumulated significantly more of the tebuthiuron and hexazinone label than did the other species at both time intervals. Tebuthiuron activity increased in the roots of bur oak and loblolly pine between the 4 and 24 h sampling period. Activity also increased with time in both black walnut and loblolly pine following ^{14}C-hexazinone treatment. The rate of increase of ^{14}C in the roots leveled off by 24 h in bur oak but was generally linear in eastern redcedar up to 96 h (data not shown).

Activity from the tebuthiuron treatment was 26 to 34 times greater in the upper and lower stems of bur oak respectively than in black walnut at 24 h (Table 4). This indicates a slower rate of movement out of black walnut roots. This reduction in movement may be a mechanism for tebuthiuron resistance by black walnut. Activity from the ^{14}C-hexazinone treatment was also greater in bur oak stems than in black walnut at 24 h, but only a 2 fold difference in activity was noted. The lower and upper stems of bur oak accumulated more of the tebuthiuron label than the hexazinone label. However, the accumulation of ^{14}C in the upper and lower stems of black walnut at 24 h was greater following treatment with ^{14}C-hexazinone than with ^{14}C-tebuthiuron.

In general, the tolerance of a plant to a photosynthetic inhibitor should be inversely related to the concentration of the intact compound in the foliage. With the exception of the loblolly treated loblolly pine seedlings, this relationship seems to hold true when comparing the concentration of ^{14}C detected in the foliage of the four older species following treatment with either ^{14}C-tebuthiuron or ^{14}C-hexazinone (Table 5). No activity was detected in the foliage of black walnut at the 4 h treatment interval with ^{14}C-tebuthiuron, and only minimal activity was detected in the foliage of the other three species. By 24 h, the susceptible (S) bur oak and the intermediate (I) loblolly pine accumulated more of the tebuthiuron label than the resistant (R) black walnut or eastern redcedar.

Initial (4 h) accumulation of the hexazinone label in the older species was similar to that of the tebuthiuron label. The amount of activity recovered in the susceptible species (bur oak and black walnut) was higher than in the more tolerant species with the exception of loblolly pine. Loblolly pine, a resistant species, accumulated more of the hexazinone label than bur oak (S), black walnut (S), or eastern redcedar (I). The large amount of activity in the foliage of loblolly pine following treatment with hexazinone may explain the early onset of phytotoxic symptoms observed in loblolly pine seedlings (3,19). South et al. (19) reported that postemergence applications of hexazinone at rates of 0.5 kg/ha and higher resulted in severe pine seedling injury. Fitzgerald and Fortson (3) observed that the needles of pine apices became necrotic 27 days after treatment under greenhouse conditions and that the injured pine seedlings either died or recovered during the first growing season.

Degradation. Degradation processes also play an important role in determining selectivity, as differing capacities to metabolize a herbicide can outweigh the effect of differential movement of the compound within them. It is likely to be the most important factor contributing to selectivity (14).

Thin layer chromatography was used to identify tebuthiuron and its metabolites in bur oak, eastern redcedar, and loblolly pine (Table 6). Free tebuthiuron, Rf of .47 to .50, was identified in the root cyclohexane fractions of bur oak and eastern redcedar at both harvest intervals. Tebuthiuron was also found in the lower and upper foliage of bur oak at the 4 and 24 h sampling periods. Tebuthiuron was not detected in loblolly pine. An unknown metabolite, Rf .46, was recovered from the 4 h ethyl acetate fraction (upper foliage) of eastern redcedar. This compound cochromatographed with the tebuthiuron reference standard; however, since this fraction was hydrolyzed with HCL, it is probably a carboxylic acid analog with a Rf value similar to tebuthiuron.[4]

One major metabolite with an Rf of .34 to .36 cochromatographed with the reference standard [N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N-methylurea] designated as compound (104). This metabolite was isolated 4 h after treatment from the upper foliage of bur oak and loblolly pine in the ethyl acetate fraction. At 24 h, compound (104) was detected in the upper foliage of the three woody species and in the upper foliage of bur oak and loblolly pine.

A second metabolite, compound (109), cochromatographed with the

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[4]Personal Communication, John Magnussen, Research Scientist, Lilly Research Laboratories.

reference standard [N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N'-hydroxymethylurea]. This metabolite was isolated from the dichloromethane fraction of the lower foliage of eastern redcedar 24 h after treatment. The formation of the N'-hydroxymethyl and N'-demethylated metabolites is a degradative pathway typically observed for substituted urea herbicides (2). Demethylation appears to be the primary detoxification mechanism of tebuthiuron by the woody species examined in this investigation.

Hexazinone and its metabolites were detected only at the 24 h sampling period in the tissue samples of loblolly pine (Table 7). Free hexazinone, Rf of .38, was identified in the root cyclohexane fraction of loblolly pine. Three degradation products of hexazinone were also detected in loblolly pine. One of these metabolites, Rf .84, was recovered from the root cyclohexane fraction, and the two remaining metabolites were isolated from the foliage. These compounds were not identified but it is believed that they represent hydroxylation or N-dealkylation derivitives of hexazinone as these are the two major pathways observed for triazine degradation in higher plants (1). The presence of the three metabolites in loblolly pine suggests that this is the mechanism imparting resistance to hexazinone since the parent compound was not detected in the foliage. Similar conclusions were reached when Lund-Hoie (10) reported that simazine resistant Norway spruce (Picea abies L.) was able to degrade simazine fast enough to prevent its accumulation in the needles.

Results of this study indicate that the selectivity of tebuthiuron and hexazinone among the species evaluated can be attributed to the amount of intact herbicide translocated to the foliage. Species more

tolerant to tebuthiuron, loblolly pine (I) and eastern redcedar (R), were able to prevent the accumulation of the parent compound in the foliage by the 24 h sampling period. Demethylation was determined to be the primary detoxification mechanism of tebuthiuron by eastern redcedar, loblolly pine, and bur oak. Loblolly pine, a hexazinone resistant species, was able to rapidly degrade hexazinone into three unknown degradation products thereby preventing its accumulation in the foliage.

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Table 1. Mean concentration of ^{14}C activity in the roots and stems of four-month old winged elm seedlings after treatment with ^{14}C-tebuthiuron and ^{14}C-hexazinone.

Treatment duration	Plant part		
	roots	lower stem	upper stem
(hours)	(nmoles/g)		
^{14}C-tebuthiuron			
4	6.01	4.56	2.75
24	8.03	10.23	8.13
^{14}C-hexazinone			
4	4.87	6.45	4.45
24	6.77	11.70	5.54

LSD (0.05)=2.27 for plant part within a fixed herbicide and time.

LSD (0.05)=3.39 for time comparison within a fixed herbicide and plant part.

Table 2. Mean concentration of ^{14}C activity in the foliage of four-month old winged elm seedlings after treatment with ^{14}C-tebuthiuron and ^{14}C-hexazinone.

Treatment duration	Leaf position[a] 1	2	3	4	5	6
(hours)	(nmoles/g)					
^{14}C-tebuthiuron						
4	0.37	0.55	0.52	0.58	0.57	0.65
24	1.57	1.99	2.18	2.48	2.58	2.31
^{14}C-hexazinone						
4	1.33	1.58	1.70	1.52	1.42	1.10
24	1.97	2.53	2.67	2.88	2.98	2.44

LSD (0.05)=0.52 to compare plant parts within a fixed herbicide and time.

LSD (0.05)=1.15 for time comparisons within a fixed herbicide and plant part.

[a]Leaf position 1 equals the top leaf, and leaf position 6 equals the bottom leaf.

Table 3. Mean concentrations of ^{14}C activity in the roots of year old tree seedlings after treatment with ^{14}C-tebuthiuron or ^{14}C-hexazinone.

Treatment duration	Black Walnut	Bur Oak	Eastern Redcedar	Loblolly Pine
(hours)	(nmoles/g)			
^{14}C-tebuthiuron				
4	2.00	3.01	1.66	12.50
24	3.25	9.93	2.72	16.85
72	--	10.39	4.40	--
^{14}C-hexazinone				
4	1.93	1.78	1.19	7.86
24	5.10	3.09	1.49	11.47
72	--	3.08	2.38	--

LSD (0.05)=2.52 for species within a fixed herbicide and time.

LSD (0.05)=2.41 for time comparison within a fixed herbicide and species.

LSD (0.05)=6.43 for herbicide comparison within a fixed species and time.

Table 4. Mean concentrations of ^{14}C activity in lower and upper stems of black walnut and bur oak after treatment with ^{14}C-tebuthiuron and ^{14}C-hexazinone.

	Lower stem		Upper stem	
Treatment duration	Black Walnut	Bur Oak	Black Walnut	Bur Oak
(hours)	(nmoles/g)			
^{14}C-tebuthiuron				
4	0.02	0.04	0.01	0.04
24	0.12	3.03	0.07	2.26
72	--	2.46	--	2.69
^{14}C-hexazinone				
4	0.04	0.06	0.04	0.07
24	0.65	1.33	0.62	1.22
72	--	1.99	--	2.32

LSD (0.05)=0.44 (lower stem) and 0.19 (upper stem) for comparison of species within a fixed herbicide and time.

LSD (0.05)=0.49 (lower stem) and 0.22 (upper stem) for comparison of time within a fixed herbicide and species.

LSD (0.05)=0.57 (lower stem) and 0.25 (upper stem) for herbicide comparison within a fixed species and time.

Table 5. Mean concentrations of ^{14}C activity in the foliage of year old tree seedlings after treatment with ^{14}C-tebuthiuron and ^{14}C-hexazinone.[a]

Treatment duration	Black Walnut	Bur Oak	Eastern Redcedar	Loblolly Pine
(hours)	(nmoles/g)			
^{14}C-tebuthiuron				
4	0.00	0.04	0.01	0.03
24	0.06	1.68	0.18	0.58
72	--	1.98	0.49	--
^{14}C-hexazinone				
4	0.00	0.07	0.05	0.05
24	0.42	0.53	0.21	2.24
72	--	0.72	0.56	--

LSD (0.05)=0.34 for species within a fixed herbicide and time.

LSD (0.05)=0.31 for time comparison within a fixed herbicide and species.

LSD (0.05)=0.36 for herbicide comparison within a fixed species and time.

[a]Only the first two leaves of the deciduous trees and the upper foliage of the coniferous trees were subjected to analysis.

Table 6. Rf values of standards and degradation products in extracts developed in hexane: acetone (60:40, v/v) following treatment with tebuthiuron.[a]

Plant		Bur Oak		Eastern Redcedar		Loblolly Pine	
Part	Fraction	(4 h)	(24 h)	(4 h)	(24 h)	(4 h)	(24 h)
Roots							
	cyclohexane	.47	.47	.48	.50		
	dichloromethane						
	ethyl acetate						
Lower foliage							
	cyclohexane	.48	.48				
	dichloromethane				.26		
	ethyl acetate		.34		.35		.35
Upper foliage							
	cyclohexane	.47	.47				
	dichloromethane						
	ethyl acetate	.35	.35	.46		.35	.36

[a]Rf values of tebuthiuron and metabolites 109 and 104 are .46-.50, .24-.26, and .34-.36 respectively.

Table 7. Rf values of standards and degradation products in extracts developed in ethyl acetate:methanol (9:1, v/v) following hexazinone treatment.[a]

Plant Part	Fraction	Loblolly Pine (24 h)
Roots		
	cyclohexane	.38, .84
	dichloromethane	
	ethyl acetate	
Lower foliage		
	cyclohexane	.72
	dichloromethane	.72
	ethyl acetate	.79
Upper foliage		
	cyclohexane	.72
	dichloromethane	
	ethyl acetate	.79

[a]The Rf value of ^{14}C-hexazinone was .38.

VITA

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Doctor of Philosophy

Thesis: FACTORS INFLUENCING THE TOXICITY OF HEXAZINONE AND TEBUTHIURON IN SEVERAL WOODY SPECIES

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