

THE ABSORPTION AND TRANSLOCATION
OF TERBUTRYN AND PROPAZINE
IN SORGHUM AND WHEAT

By

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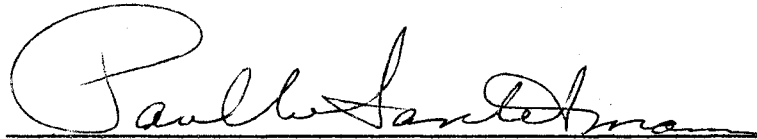
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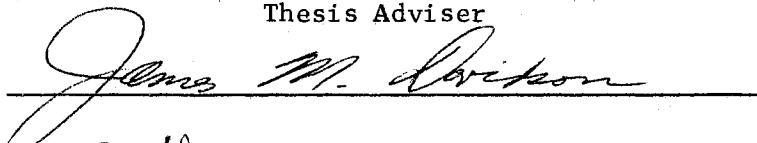
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Thesis Approved:



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TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. LITERATURE REVIEW	3
III. METHODS AND MATERIALS	14
IV. RESULTS AND DISCUSSION	21
V. SUMMARY	46
LITERATURE CITED	48

LIST OF TABLES

Table	Page
I. Visual Rating (0-10 Scale) of the Susceptibility of Various Grass Species to Terbutryn	22
II. Accumulation of ^{14}C -Terbutryn in Sorghum Plant Parts (nanograms ^{14}C -herbicide/g fresh plant tissue)	25
III. Accumulation of ^{14}C -Terbutryn in Wheat Plant Parts (nanograms ^{14}C -herbicide/g fresh plant tissue)	28
IV. Accumulation of ^{14}C -Propazine in Sorghum Plant Parts (nanograms ^{14}C -herbicide/g fresh plant tissue)	33
V. Accumulation of ^{14}C -Propazine in Wheat Plant Parts (nanograms ^{14}C -herbicide/g fresh plant tissue)	35
VI. Accumulation of ^{14}C -Terbutryn in Sorghum Plant Parts Under Different Temperatures (nanograms ^{14}C - herbicide/g fresh plant tissue)	38
VII. Accumulation of ^{14}C -Terbutryn in Wheat Plant Parts Under Different Temperatures (nanograms ^{14}C - herbicide/g fresh plant tissue)	39
VIII. Accumulation of ^{14}C -Propazine in Sorghum Plant Parts Under Different Temperatures (nanograms ^{14}C - herbicide/g fresh plant tissue)	40
IX. Accumulation of ^{14}C -Propazine in Wheat Plant Parts Under Different Temperatures (nanograms ^{14}C - herbicide/g fresh plant tissue)	40
X. Chromatography of Terbutryn in Sorghum (nanograms herbicide)	42
XI. Chromatography of Terbutryn in Wheat (nanograms herbicide)	42

LIST OF FIGURES

Figure	Page
1. Plant Sectioning of a Sorghum Plant After Exposure to ^{14}C -Terbutryn or ^{14}C -Propazine Applied Through the Roots	17
2. Plant Sectioning of a Wheat Plant After Exposure to ^{14}C -Terbutryn or ^{14}C -Propazine Applied Through the Roots	18
3. Response of Sorghum, Wheat, Oats, Barnyardgrass, and Crabgrass to Varying Terbutryn Concentrations	23
4. ^{14}C -Terbutryn Absorption and Translocation in Sorghum at Various Time Intervals After Treatment	26
5. ^{14}C -Terbutryn Absorption and Translocation in Wheat at Various Time Intervals After Treatment	29
6. Autoradiogram of Sorghum Plant After 12 Hours Exposure to ^{14}C -Terbutryn Applied Through the Roots	30
7. Autoradiogram of Sorghum Plant After 48 Hours Exposure to ^{14}C -Terbutryn Applied Through the Roots	30
8. Autoradiogram of Wheat Plant After 48 Hours Exposure to ^{14}C -Terbutryn Applied Through the Roots	31
9. ^{14}C -Propazine Absorption and Translocation in Sorghum at Various Time Intervals After Treatment	34
10. ^{14}C -Propazine Absorption and Translocation in Wheat at Various Time Intervals After Treatment	37

CHAPTER I

INTRODUCTION

Herbicides have proven themselves effective for controlling undesirable vegetation in crops; thus expressing selectivity. However, in some instances, crops may be injured by the herbicide. There seem to be many plant factors correlated with herbicide resistance and susceptibility. Determination of the absorption, translocation, and accumulation patterns of a herbicide and/or metabolite may help explain resistance and susceptibility. Therefore, an understanding of the absorption and translocation characteristics of a herbicide within the plant may help reduce the possibility of crop damage. The use of radioactive tracers is probably one of the most frequently used methods of tracing a material as it moves throughout a plant. By using tracers, it is possible to determine the distance and location to which a given chemical and/or metabolite has been transported in the plant.

Terbutryn [2-(tert-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine] is an effective herbicide for the control of broadleaf weeds and grasses in winter wheat (Triticum aestivum L.) and grain sorghum (Sorghum bicolor L.). However, under certain conditions, herbicide injury to the crop has been reported.

The objectives of this study were: (1) to determine terbutryn susceptibility and resistance in plant species under defined conditions, (2) to establish absorption and translocation patterns of terbutryn in

specific plant species, (3) to determine the influence of different temperatures on terbutryn absorption and translocation, and (4) to determine whether ^{14}C -radioactivity in certain regions of the plant is associated with the parent chemical or a metabolic product. Propazine [2-chloro-4,6 bis (isopropylamino)-s-triazine], which is a related s-triazine herbicide, was used as a standard for comparison in the ^{14}C -labeled herbicide studies.

CHAPTER II

LITERATURE REVIEW

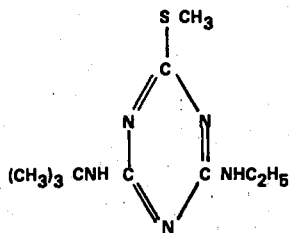
Terbutryn and propazine are related s-triazines with the chemical structures and physical properties shown on the following page.

The literature describing physiological studies with terbutryn is negligible. Studies conducted with other s-triazines, especially the chloro-triazines, will therefore be used for information concerning possible terbutryn activity.

Hammerton (13) has reviewed the literature concerning environmental factors influencing plant susceptibility to herbicides. There are many factors that can influence herbicidal activity and any one factor can be the critical determinant. Temperature, humidity, and light intensity rank among the major factors influencing absorption and translocation of herbicides in plants.

Temperature Effects

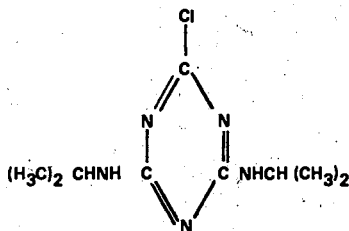
Temperature has been shown to have a pronounced effect upon a plant's response to a herbicide. Houseworth and Tweedy (15) found that when the temperature was raised from a low level (19°C day, 14°C night) to a high level (29°C day, 24°C night), terbutryn toxicity was increased 1.6 times for oats (Avena sativa L.) and 1.3 times for cucumber (Cucumis sativus L.). One of their conclusions was that environmental conditions favoring rapid growth resulted in an increase



Terbutryn

Terbutryn [2-(tert-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine]

Molecular weight-----241
 Melting point-----104-105 C.
 Acute oral toxicity (LD₅₀) in rats-----2980 mg/kg
 Physical state and color-----White, crystalline
 Solubility in water-----58 ppmw at 20 C



Propazine

Propazine [2-chloro-4,6 bis(isopropylamino)-s-triazine]

Molecular weight-----229.7
 Melting point-----212-214 C.
 Acute oral toxicity (LD₅₀) in rats-----5000 mg/kg
 Physical state and color-----Colorless, crystalline
 Solubility-----8.6 ppmw at 20 C

in phytotoxicity. Figuerola and Furtick (9) grew wheat plants at 15°C for 1 week and then exposed the plants to temperatures of 20°C or 5°C for 48 hours before terbutryn treatment. Plants at the 20°C temperature developed injury symptoms in 3 or 4 days and showed severe injury symptoms after 2 weeks, whereas the 5°C exposed plants showed only slight chlorosis and stunting. Wax and Behrens (44) reported that there was a greater uptake and translocation of foliar applied atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] in quackgrass [Agropyron repens (L.) Beau.] when the temperature was increased from 15° to 27°C. Sheets (33) in a classical study on the uptake and distribution of simazine [2-chloro-4,6-bis(ethylamino)-s-triazine] in oats (very susceptible) and cotton (Gossypium hirsutum L.) (intermediate in susceptibility) seedlings revealed a rapid distribution of ¹⁴C-simazine in the oat plant. There was greater absorption and translocation of radioactivity at 37°C than at 26°C. Vostral et al. (43) reported that root temperature had more influence on atrazine accumulation in soybean [Glycine max (L.) Merrill] stems and leaves than in roots. Noda and Ibaraki (27) reported that prometryne's [2,4-bis(isopropylamino)-6-(methylthio)-s-triazine] phytotoxicity to rice (Oryza sativa L.) seedlings was influenced more by a high air temperature than soil temperature. Ibaraki (16) found that the phytotoxicity of cucumber seedlings to prometone [2,4-bis(isopropylamino)-6-methoxy-s-triazine] increased with a rise in temperature, in herbicide concentration in the nutrient solution, and in length of exposure of the plant to the herbicide and light. Temperature seemed to have the greatest effect and raising the temperature from 16° to 26°C resulted in more plant injury than increasing the herbicide concentration from 4 x 10⁻⁶ to

8×10^{-6} M. Muzik (25) evaluated the effect of temperature on uptake of atrazine and several other herbicides by wheat roots and concluded that reduced root uptake due to low temperatures may be an important factor in reducing herbicide damage to wheat.

Pine trees (*Pinus resinosa* Ait.) seem to be influenced by herbicides when temperature increases. Kozlowski et al. (17) treated pine seedlings with several s-triazine herbicides at temperatures of 5° to 30°C and concluded that high temperatures greatly accelerated herbicide activity, but the effects of temperature varied with each herbicide. Atrazine and simazine exhibited the most toxicity of all herbicides over the temperature range study.

There are many theories regarding why temperatures affect herbicide absorption and translocation within plants. Increased transpiration of treated plants at high temperatures would explain at least part of the increased absorption and translocation in plants. Sheets (33) found a trend toward greater quantities of simazine in oat leaves than cotton leaves per unit of water transpired. At high temperatures, the plant is actively transpiring and therefore the triazine herbicide, which is carried through the transpiration stream, can quickly and quantitatively be effective in the plant. Atrazine has been found to reduce transpiration in plants (40, 46).

Increased herbicidal toxicity at high soil temperatures can also be caused by a decrease in herbicide adsorption by soil and therefore make the herbicide more readily available for plant uptake (19, 42). Buchanan and Rodgers (4) reported several s-triazine herbicides can become inactivated at high temperatures. Inactivation of herbicides was studied by measuring increases in dry weight of cucumber, oats, or

soybeans grown in treated soil in response to changes in temperature or length of time from soil treatment to planting.

Relative Humidity Effects

Relative humidity seems to have a particularly pronounced effect on plant absorption and translocation of the triazine herbicides. Sheets (33) found that with a constant temperature of 37°C, the concentration of simazine in the leaves of root-fed cotton and oats was lower under a 66% relative humidity than a 33% relative humidity environment. Similarly, Wax and Behrens (44) found the amount of radioactive atrazine in quackgrass leaves decreased as relative humidity increased. This would tend to indicate greater atrazine uptake as conditions become more conducive for transpiration, suggesting movement of atrazine in the transpiration stream of the plant. This concept also appears to be born out for foliar-applied herbicides. Downward movement of herbicides appear to occur best under conditions of high humidity. Pallas (28) observed greater absorption and translocation of 2,4-D[(2,4-dichlorophenoxy) acetic acid] from leaves to hypocotyl of beans (Phaseolus vulgaris L.) plants occurred at about 70% relative humidity compared to approximately 40% relative humidity. Basler et al. (3) found greater translocation of stem-injected 2,4,5-T[(2,4,5-trichlorophenoxy) acetic acid] in bean plants at high relative humidity. At 96% relative humidity, about 40% of the total 2,4,5-T applied was detected in the nutrient solution 48 hours after treatment while there was only 23% detected in the nutrient solution at 20% relative humidity. In contrast, low humidity enhanced acropetal translocation. At 20% relative humidity, ten times more 2,4,5-T was

translocated to the primary leaves and growing point of the plant than at 96% relative humidity. Prasad et al. (29) reported a greater amount of foliar-applied dalapon (2,2-dichloropropionic acid) absorbed and translocated in barley (Hordeum vulgare L.), bean, and three other plant species at a high (88%) than a medium (60%) or low (30%) post-treatment relative humidity.

Relative humidity of the microclimate will affect herbicidal penetration (13). Elle (8) states there are three reasons why relative humidity may be important to herbicidal performance: (1) the length of time sprays remain in a liquid form on the leaf is influenced by humidity, (2) if stomates influence absorption of 2,4-D, then humidity could indirectly retard or accelerate absorption, (3) if cuticular development influences absorption, then humidity could indirectly affect absorption. Physiologically, the relative humidity will affect plants' water stress, stomatal opening, and cuticular permeability (7). Prasad et al. (29) states that the rate of droplet drying and stomatal distribution and behavior apparently contributed to the relative humidity effect.

Light Intensity Effects

Many investigators have found that light intensity can affect herbicide translocation and absorption in plants. Ashton (2) reported that the degree of injury by atrazine and monuron [3-(p-chlorophenyl)-1,1-dimethylurea] in oats and red kidney beans was influenced by light intensity; the higher the light intensity, the greater the injury. The action spectrum indicated that chlorophyll was the principal absorbing pigment involved in this injury. Figuerola and Furtick (9)

grew wheat plants under different light intensities from a low intensity (800 fc) to medium and high light intensity (1500-2200 fc) and found only slight terbutryn injury at low light intensity while medium and high light intensities showed marked symptoms. Photosynthesis was inhibited much earlier by terbutryn at high than at low light intensities. Houseworth and Tweedy (15) also showed that toxicity of soil applied terbutryn to cucumber and oats was increased 2.2 times when the light intensity was raised from 11,000 to 22,000 lumens/m².

Herbicide injury to plants caused by low light intensity appears to be associated with carbohydrate content of the plant (13). Mitchell and Brown (21) found that translocation of 2,4-D from leaf tissue is correlated with carbohydrate level. Weaver and DeRose (45) found 2,4-D readily moved downward whenever translocation of synthesized materials occurred. Rice (30) added sucrose to a 2,4-D spray solution and obtained increased plant response. Rohrbaugh and Rice (31) increased the carbohydrate content of plants exposed to total darkness by immersing leaves in a spray solution and were successful in obtaining responses from 2,4-D.

Miscellaneous Effects

Minshall (20) reported that when he applied potassium nitrate or urea to the soil of detopped potted tomato (Lycopersicon esculentum Mill.) plants, there was an increase in the rate of exudation from the stumps of the plants from 100 to over 300% and increased the concentration of atrazine in this augmented exudate from 9 to 40%. Applications of certain metabolites, especially thiamin, increased the sensitivity of fiddleneck (Amsinckia spp) to 2,4-D at low temperatures.

Chen and Ries (5) found oats grown in a nitrate-substrate in a growth chamber showed a rapid decrease in nitrate content and subsequent nitrate reductase activity, whereas, plants receiving 3.2 μM of simazine showed no appreciable decrease in either during a 6 day period. Oats grown with potassium nitrate as a source of nitrogen were injured more rapidly and severely than those grown on ammonium sulfate. The toxicity was associated with high nitrate and free ammonium levels in oat plants. The effect of simazine on nitrate content, nitrate reductase activity, and subsequently higher levels of toxic ammonia suggest the toxic action of simazine in vivo may be linked with nitrogen metabolism.

Mode of Action

Many of the s-triazine herbicides have proven themselves as preemergent or post-emergent treatments. Terbutryn can be utilized by either method (1). Atrazine seems to be equal to simazine as a pre-emergent chemical in corn (Zea mays L.), yet it demonstrates excellent contact kill of small seedling weeds shortly after emergence (32).

When a plant is exposed to a triazine herbicide, the chemical rapidly enters the stele of the root and migrates to the apoplast and then moves in the xylem (6). This apoplastic movement is accelerated by the flow of water and therefore often results in a wedge-shaped pattern of distribution in leaf tissue. These compounds are absorbed rapidly and they must pass across the symplast in the endodermous layer. This indicates that living cells of the root are much less susceptible to triazine injury than cells of the leaf. Guttation experiments suggest that triazines are taken up and translocated in the xylem in the transpiration stream (6). Triazine herbicides that enter the living

cells of the chlorenchyma rapidly brings about drastic changes in metabolism as evidenced by a rapid blockage of photolysis of water and evolution of oxygen (12). Glucose supplied to barley plants through severed leaf tips helped keep plants alive and growing in the presence of otherwise lethal concentrations of simazine. Shimabukuro and Swanson (36) found atrazine to readily penetrate the chloroplast of resistant as well as susceptible plants and seemed to accumulate there until an equilibrium concentration was attained between the chloroplasts and the cytoplasm. They concluded in resistant plants, such as sorghum, metabolism of atrazine probably occurs outside the chloroplast to form a water-soluble residue, reducing the concentration of the photosynthetic inhibitor in the chloroplasts, and this results in a partial recovery of photosynthesis.

Smith and Buchholtz (41) as well as Wills et al. (46) found that triazine herbicides induce stomatal closure. In herbicide-treated plants, the severe injury at high temperatures apparently is related largely to absorption of large amounts of herbicides. Passive movement of triazine herbicides to shoots is probably related to the volume of water transpired.

One possibility that may cause differences in plant susceptibility among herbicides is related to the amount of herbicide absorbed and translocated to vital target areas within the plant system. Sheets (33) found approximately 3 times more simazine and/or degradation products in the leaves of oat plants (very susceptible) than cotton (intermediate in susceptibility) when the plants were treated similarly. Sikka and Davis (39) found 4 times more prometryne in the shoots of soybeans (sensitive) than cotton (tolerant). However, cotton had a

slightly higher percentage of the nonphytotoxic derivate in the shoots than soybeans. He concluded differential translocation of prometryne appeared to be a major factor contributing to the differences in susceptibility. Conversely, many workers have shown that resistance of plants to triazine herbicides was related to their power to degrade them to nonphytotoxic metabolites. Montgomery and Freed (23) believe that in most cases, the resistance of plants to the triazine herbicides cannot be explained by the amount of chemical absorbed. Shimabukuro (35) found the rate and pathway of atrazine metabolism are important in determining the tolerance of plants to atrazine. Also, both quantitative and qualitative differences in atrazine metabolism was detected between resistant, intermediate in susceptibility, and susceptible plant species. Norway spruce (Picea abies (L.) Karst.), which has a high tolerance to simazine, degraded simazine in the roots and stem and only metabolites could be found in the needles (18). Montgomery and Freed (22) found corn degraded simazine to metabolites with time. Negri et al. (26) reported that atrazine absorption was not directly related with plant susceptibility but the amount of hydroxyatrazine was correlated. Also, more susceptible plant species had a lower phenol content. Cotton seems to accumulate prometryne or metabolites in the lysigenous glands of stem, leaves, and root primordia which was thought to aid in tolerance to the chemical (26,39). Other workers have shown that the lysigenous glands play no part in increasing atrazine tolerance in cotton (37). In any event, the problems of completely solving the question of tolerance and susceptibility has not been overcome. A relative new pathway of atrazine detoxification in corn has been reported by Shimabukuro et al. (38) in

which glutathione conjugation was found to be a major detoxification mechanism in corn leaf tissue but not when atrazine was absorbed through the roots.

In reviewing the literature, there appears to be many complex factors and interactions among factors that can influence the degree of susceptibility or resistance to herbicides among plant species. This study was conducted to attempt to determine why sorghum was resistant to terbutryn but wheat was susceptible. A study of the translocation and absorption patterns was initiated and an attempt was made to correlate species susceptibility and resistance to translocation.

CHAPTER III

METHODS AND MATERIALS

Preliminary Bioassay Studies

Growth chamber studies were conducted to determine the best bioassay species to use as terbutryn resistant and susceptible species with radioactive tracer procedures. Commercially formulated 80% active ingredient, wettable powder, terbutryn was applied to an air-dried sandy loam soil and mixed for 5 minutes in a V-type soil blender. The treated soil was then serially diluted with similar untreated soil to give terbutryn concentrations of 0, 0.5, 1, 2, and 4 ppmw. Wheat (variety Kaw), oats (variety Cimmaron), sorghum (variety OK 612), crabgrass (Digitaria sanguinalis (L.) Scop.), and barnyardgrass (Echinochloa spp) were evaluated as bioassay plants. Approximately 200 grams of treated soil was placed in each 6 ounce styrofoam cup prior to planting. The cups were then placed in the growth chamber with an alternating 14 hr daylength at 32°C and 10 hrs of dark at 27°C with a 40 ± 10% relative humidity. The light source consisted of incandescent and fluorescent lamps supplying approximately 2500 fc at soil level. There were four replications per treatment in a completely randomized design. One week after planting, the species were thinned to 10 plants per cup. Herbicidal response of the plant species was measured by:

- (1) visual rating based on a 0 to 10 scale with 0 representing no plant

injury scaling to 10 indicating complete death of the plant and (2) harvesting the plants 17 days after planting, drying them in an oven at 90°C for 24 hrs, and obtaining the dry weight. Dead plants were represented as having no dry weight.

Another experiment consisted of obtaining the lower limits of species tolerance by diluting the herbicide concentration to 0, $\frac{1}{2}$, $\frac{3}{4}$, 1, and 2 ppmw. The same procedure was followed as previously mentioned.

General Procedures for Radioactive Tracer Studies

Experiments were conducted to determine the absorption and translocation patterns of labeled terbutryn and propazine in wheat and sorghum. Sorghum and wheat seeds were germinated in paper rolls at 32°C for 7 days and then transferred to nutrient jars containing 70 ml of $\frac{1}{2}$ strength Hoaglands nutrient solution. The nutrient solution was continuously aerated. The nutrient jars were wrapped in aluminum foil to eliminate algae growth and also to prevent possible photodecomposition of the herbicide. The plants were placed in a growth chamber under an alternating 14 hr daylength at 32°C and 10 hours of dark at 27°C with a $40 \pm 10\%$ relative humidity. Sorghum was treated 14 days and wheat 18 days after planting by direct root exposure to the treated nutrient solutions. Treatments consisted of 0.4 μM (or 6.74 ug/70 ml) solutions of uniform ^{14}C -ring labeled terbutryn (specific activity 7.9 $\mu\text{C}/\text{mg}$) or a 0.4 μM (or 6.42 ug/70 ml) solutions of uniform ^{14}C -ring labeled propazine (specific activity 9.2 $\mu\text{C}/\text{mg}$). Plants in the treated nutrient jars were placed under continuous light and harvested at various time intervals. The roots were washed under tap water for 15 seconds to eliminate part of the radioactivity adhering to the root surfaces when harvesting.

Plants were sectioned into roots, stem, youngest leaf, 2nd leaf, oldest leaf, and then the fresh weight of each part determined (Figures 1 and 2). All plants were then quickly frozen for storage at -40°C and the radioactivity determined later. Radioactivity was determined for each plant part by grinding the plant part with 10 ml of 95% ethanol in a Virtis homogenizer. A 0.5 ml portion of the homogenate was placed in a counting vial containing 15 ml of counting solution and counted in a Beckman liquid scintillation counter, model LS-100. Counts were corrected for background and quench. The counting solution consisted of xylene, p-dioxane, and ethanol (5:5:3 v/v) containing 80 grams of naphthalene and 5 grams of PPO per liter of solution. There were 4 replications per harvest time in a completely randomized design. Each experiment was duplicated.

Temperature Effects

The effects of different temperatures were investigated by treating sorghum and wheat and placing them in a growth chamber at 16°C , 24°C , or 32°C . The treated plants were placed under continuous light at one of the temperatures, harvested 24 hrs after treatment, and absorption and translocation patterns determined by liquid scintillation counting.

Autoradiography

Autoradiograms of whole plants were prepared as an aid in the study of the translocation and absorption of labeled terbutryn in sorghum and wheat. Experiments were conducted using the same procedures as described previously for radioactive tracer studies but the

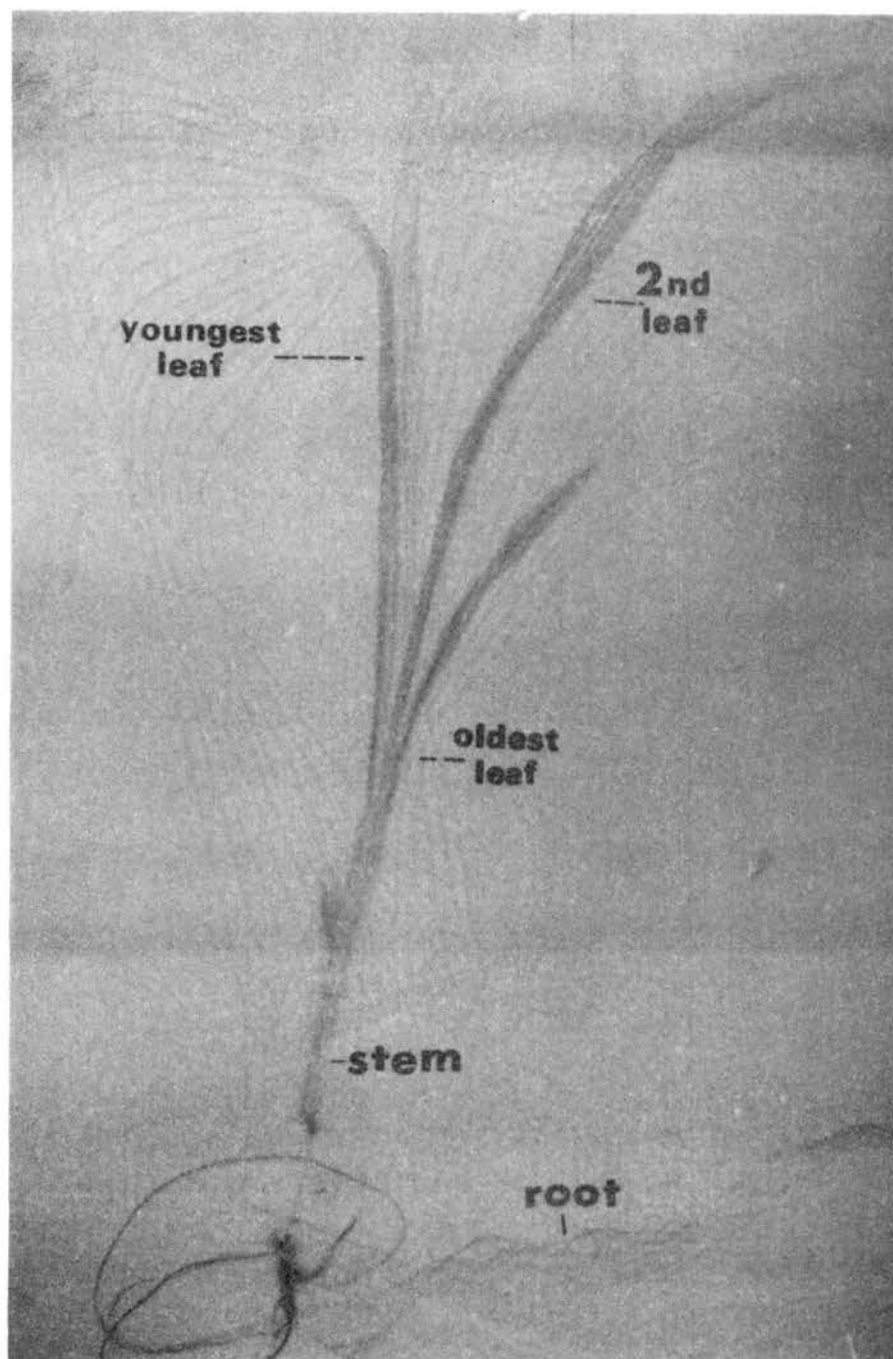


Figure 1. Plant Sectioning of a Sorghum Plant After Exposure to ^{14}C -Terbutryn or ^{14}C -Propazine Applied Through the Roots.

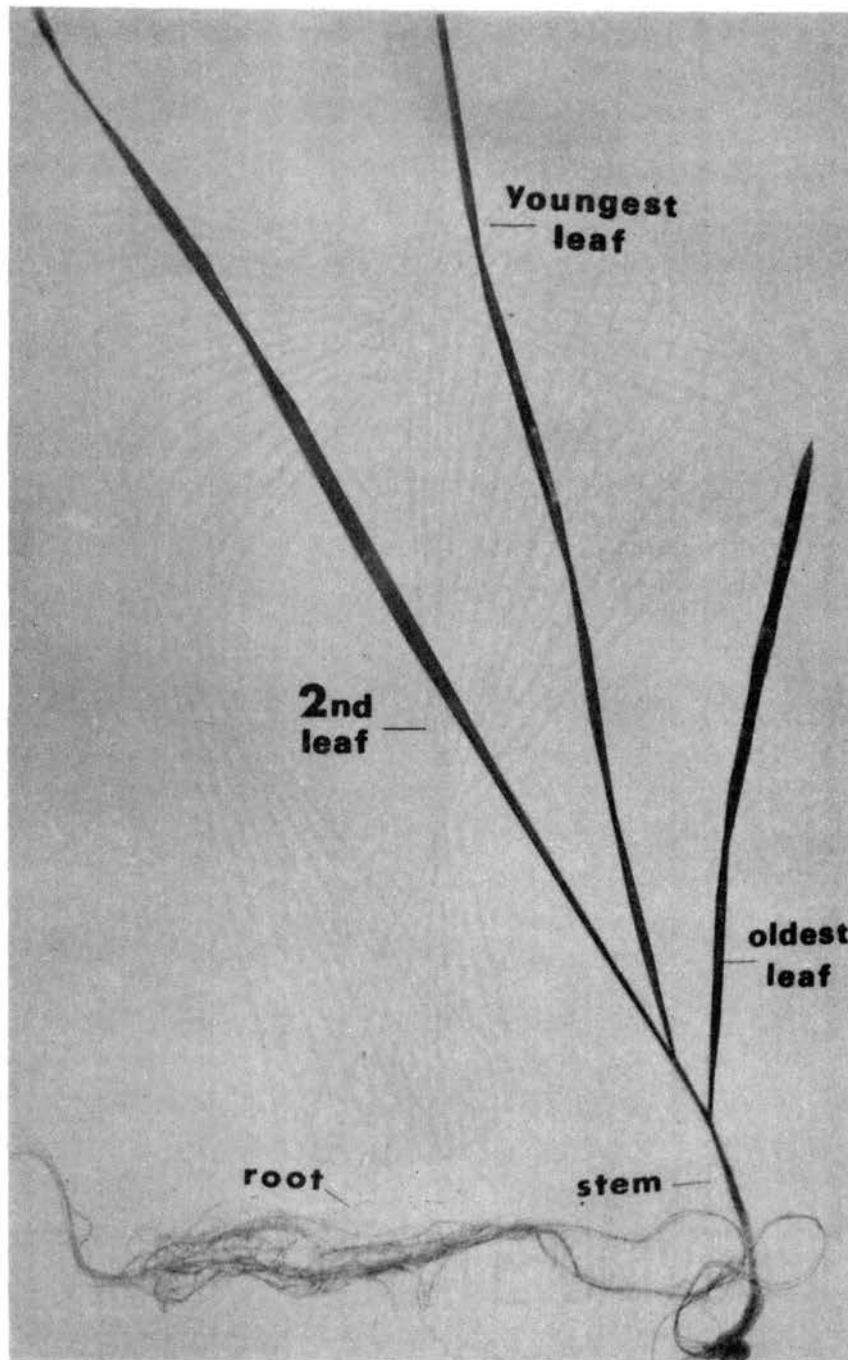


Figure 2. Plant Sectioning of a Wheat Plant After Exposure to ^{14}C -Terbutryn or ^{14}C -Propazine Applied Through the Roots.

plants were harvested 12, 24, and 48 hrs after treatment. The plants were mounted on white cardboard and covered with plastic (Saran) wrap. In a dark room, the mounted plants were placed in a film packet of Kodak Ready Pack No-Screen X-Ray film and sealed with masking tape. The film packet was then placed between a piece of sponge rubber and plywood covered with aluminum foil. A stack of film packets, sponge rubber, and plywood was formed and bound together in a plant press by 2 cotton web belts. The plants were then kept frozen at -40°C until the film was developed 45 days after the first treatment.

Thin-Layer Chromatography

In order to determine whether the radioactivity counted in various parts of the plant was the parent chemical or a metabolic product, the various plant parts were homogenized in 10 ml of 95% ethanol, evaporated to a small volume by placing in a stream of dry nitrogen, and spotted on a Silica SP-1F thin-layer chromatogram. Developing solvent consisted of benzene, chloroform, and ethyl acetate (40:40:20 v/v). The solvent front was allowed to move approximately $2\frac{1}{2}$ hrs. The chromatograms were then air dried for 12 hrs and placed in a packet of Kodak Ready Pack No-Screen X-Ray film to locate the various radioactive sites. After the sites were located, the radioactive locations were cut from the chromatogram and counted by liquid scintillation to confirm and quantify the radioactivity. A comparison of the stock solution of labeled terbutryn and the plant part homogenate was used to determine whether the parent chemical and/or metabolic product was being translocated to the various plant parts.

Statistical Analysis

Data from the liquid scintillation experiments was analyzed by using an IBM computer, model 360. Various types of programs were modified according to the particular experiment. A standard F test and/or the Duncans Multiple Range statistical testing at the 0.05 level was utilized to test significant differences within a particular experiment.

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Bioassay Studies

Various grass species were evaluated as to their resistance or susceptibility to terbutryn. The two bioassay studies were combined. Wheat, crabgrass, and barnyardgrass were killed at herbicide concentrations greater than 2 ppmw (Table I). Oats exhibited somewhat more resistance but were moderately susceptible to terbutryn. Sorghum showed the most resistance of all plant species tested.

The average dry weight per plant at the various terbutryn concentrations showed that barnyardgrass and crabgrass were the most susceptible of all plant species studied and developed herbicide injury symptoms even at the lowest concentration of herbicide (Figure 3). Oats exhibited somewhat more tolerance to terbutryn but were chlorotic and plant height was reduced at herbicide concentrations greater than 1 ppmw. Wheat was susceptible to terbutryn at herbicide concentrations as low as 0.5 ppmw. Plant leaf tips were chlorotic and plant height was reduced at this concentration. Chlorosis was evident at all herbicide concentrations greater than 0.5 ppmw. Sorghum exhibited the most resistance to terbutryn and its range of tolerance exceeds 4 ppmw. A very small amount of plant height reduction was observed at the 4 ppmw concentration. No chlorosis or other herbicide injury symptom was

TABLE I
 VISUAL RATING (0-10 SCALE) OF THE SUSCEPTIBILITY
 OF VARIOUS GRASS SPECIES TO TERBUTRYN

Species	Terbutryn Conc. (ppmw)							
	0	0.5	0.75	1.0	1.5	2.0	3.0	4.0
Sorghum	0	0	0	0	0	0	0	1
Wheat	0	4	8	8	9	9	10	10
Oats	0	0	2	2	4	9	9	9
Barnyard Grass	0	5	6	8	8	9	10	10
Crabgrass	0	2	4	5	8	9	10	10

observed at the other terbutryn concentrations in sorghum.

Sorghum was chosen as a resistant and wheat as a susceptible plant specie for the radioactive tracer studies with labeled terbutryn. Sorghum was selected because of its resistance to terbutryn at herbicide concentrations greater than 4 ppmw. Wheat showed susceptibility at terbutryn concentrations as low as 0.5 ppmw.

Labeled Terbutryn Tracer Studies

Statistical analysis showed the duplicate experiments with terbutryn were not significantly different from each other; therefore, the two experiments were combined. Values of radioactivity are an average of 8 plants per time period.

There was an interaction of species with harvest period and plant

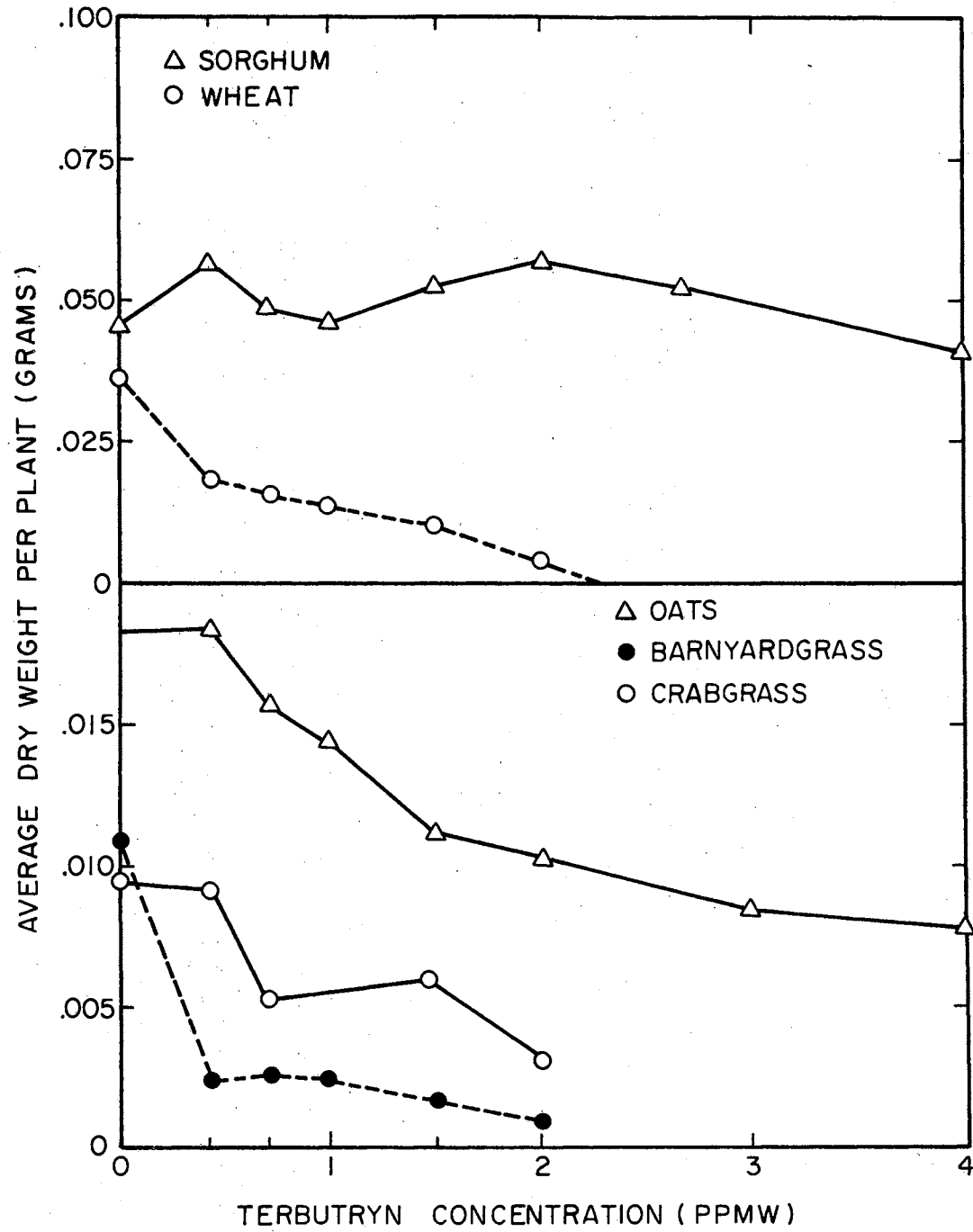


Figure 3. Response of Sorghum, Wheat, Oats, Barnyardgrass, and Crabgrass to Varying Terbutryn Concentrations

part in response to terbutryn. Thus the species did not respond to terbutryn the same. This was probably caused by different rates of absorption and translocation of the radioactivity per unit time to the various plant parts of each specie. Therefore, each specie and each plant part among each specie were analyzed separately.

Terbutryn Treatment Time Absorption and Translocation in Sorghum

The absorption and translocation patterns of terbutryn in sorghum were obtained by treating each plant with terbutryn, harvesting at specific time intervals, and determining radioactivity accumulation in each plant part. Foliage and stems contained very little radioactivity at the early harvest periods (Table II). However, radioactivity in the roots was very pronounced (Figure 4). Radioactivity increased in the foliage of sorghum in a linear manner with time after initial treatment. At the last harvest period, the older leaves contained the most radioactivity of the foliage.

The roots exhibited a different pattern of radioactivity accumulation than the other plant parts. Root radioactivity accumulation seemed to reach a peak at the 4 hour exposure time. However, sorghum roots contained the most radioactivity of all plant parts evaluated during the harvest periods.

Stem radioactivity content was large at the first few harvest periods.

TABLE II
 ACCUMULATION OF ¹⁴C-TERBUTRYN IN SORGHUM PLANT PARTS
 (NANOGRAMS ¹⁴C-HERBICIDE/g FRESH PLANT TISSUE)

Plant Part	Time After Trmt. (Hours)					
	1	2	4	8	12	24
Youngest Leaf	28.53 b*	90.81 b	130.15 b	230.22 b	362.54 b	805.57 bc
Second Leaf	56.57 b	82.72 b	144.54 b	265.92 b	375.92 b	999.71 ab
Oldest Leaf	84.90 b	101.69 b	140.85 b	288.37 b	466.57 b	966.25 bc
Stem	72.78 b	152.22 b	267.34 b	409.56 b	502.61 b	749.27 c
Roots	795.83 a	985.18 a	1342.62 a	1296.83 a	1276.04 a	1224.46 a

*Values within each time period followed by the same letter are not significantly different at the 0.05 level.

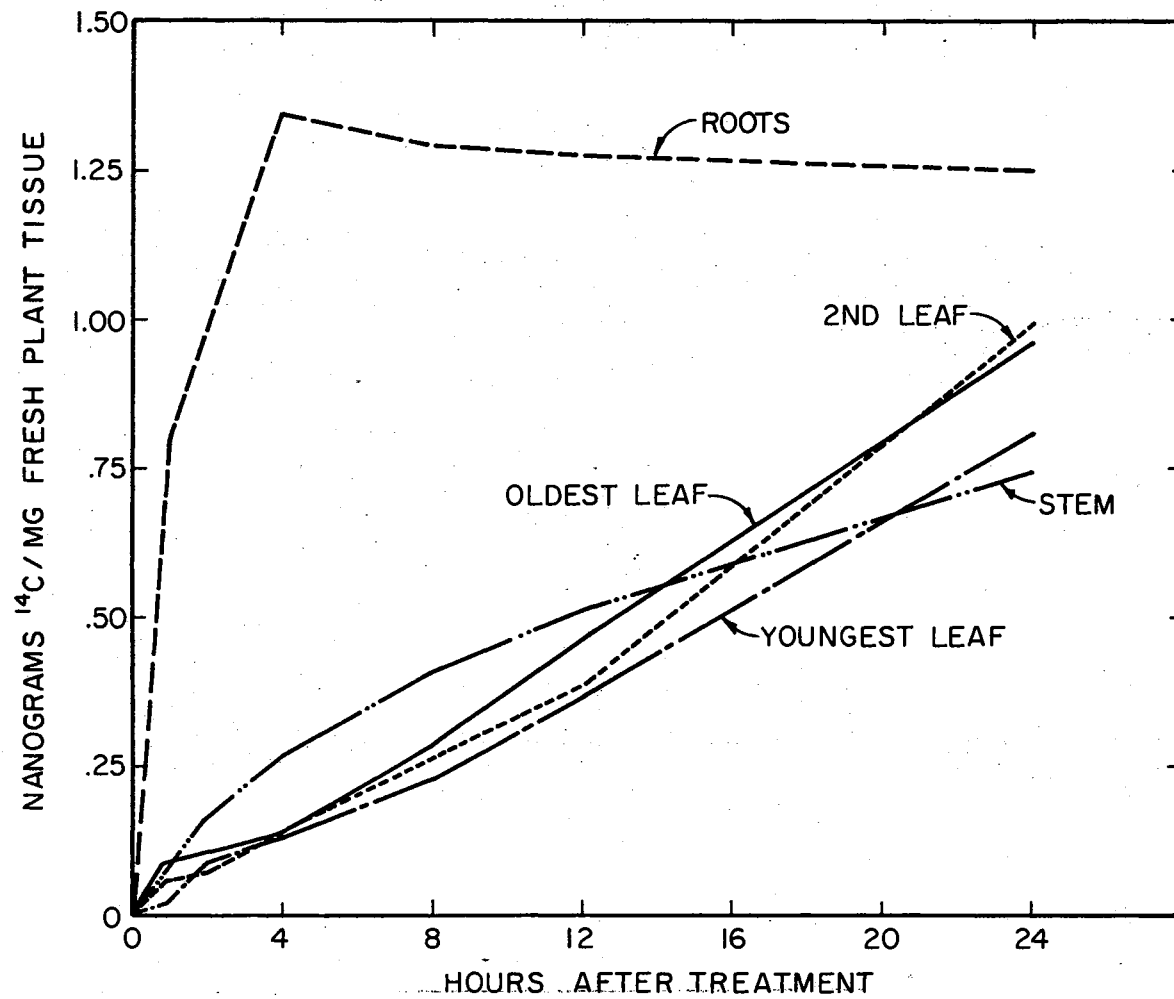


Figure 4. ^{14}C -Terbutryn Absorption and Translocation in Sorghum at Various Time Intervals After Treatment

Terbutryn Treatment Time Absorption and Translocation in Wheat

The absorption and translocation patterns of labeled terbutryn in wheat were obtained by harvesting plants at various time intervals after each initial treatment. The roots and stem contained the most radioactivity of all plant parts at the early harvest periods (Table III and Figure 5). This pattern of radioactivity accumulation occurred up to the 4 hour time period. The foliage steadily accumulated radioactivity and contained the most radioactivity at the last harvest period. Older leaves contained the most radioactivity of all plant parts studied, while the stem and roots had the least radioactivity.

Autoradiography Studies With Terbutryn

Autoradiography procedures confirmed the data obtained by the liquid scintillation procedures. Twelve hours after treatment, most of the radioactivity was confined to the roots of sorghum with very little translocation to the stem and foliage (Figure 6). Absorption and translocation of radioactivity from sorghum roots to the stem and foliage after 48 hours exposure to terbutryn was more pronounced than the earlier 12 hour treatment time (Figure 7).

Wheat absorbed and translocated very little radioactivity 12 hours after terbutryn treatment. However, radioactivity accumulation became more prominent at 48 hours (Figure 8). The autoradiography study corresponds to the data obtained from liquid scintillation counting in that sorghum accumulates the most radioactivity in the roots while foliage proved to be the major site of radioactivity accumulation in wheat.

TABLE III
 ACCUMULATION OF ¹⁴C-TERBUTRYN IN WHEAT PLANT PARTS
 (NANOGRAMS ¹⁴C-HERBICIDE/g FRESH PLANT TISSUE)

Plant Part	Time After Trmt. (Hours)					
	1	2	4	8	12	24
Youngest Leaf	89.68 c*	118.77 b	311.97 b	582.47 b	965.87 b	2007.40 b
Second Leaf	72.57 c	129.07 b	345.06 b	754.89 a	1243.96 a	2221.72 b
Oldest Leaf	84.08 c	159.35 b	423.58 a	869.04 a	1242.81 a	2625.88 a
Stem	152.26 b	265.43 a	416.61 a	471.79 b	619.82 c	804.12 c
Roots	263.06 a	246.42 a	299.23 b	321.12 c	347.87 d	504.93 d

*Values within each time period followed by the same letter are not significantly different at the 0.05 level.

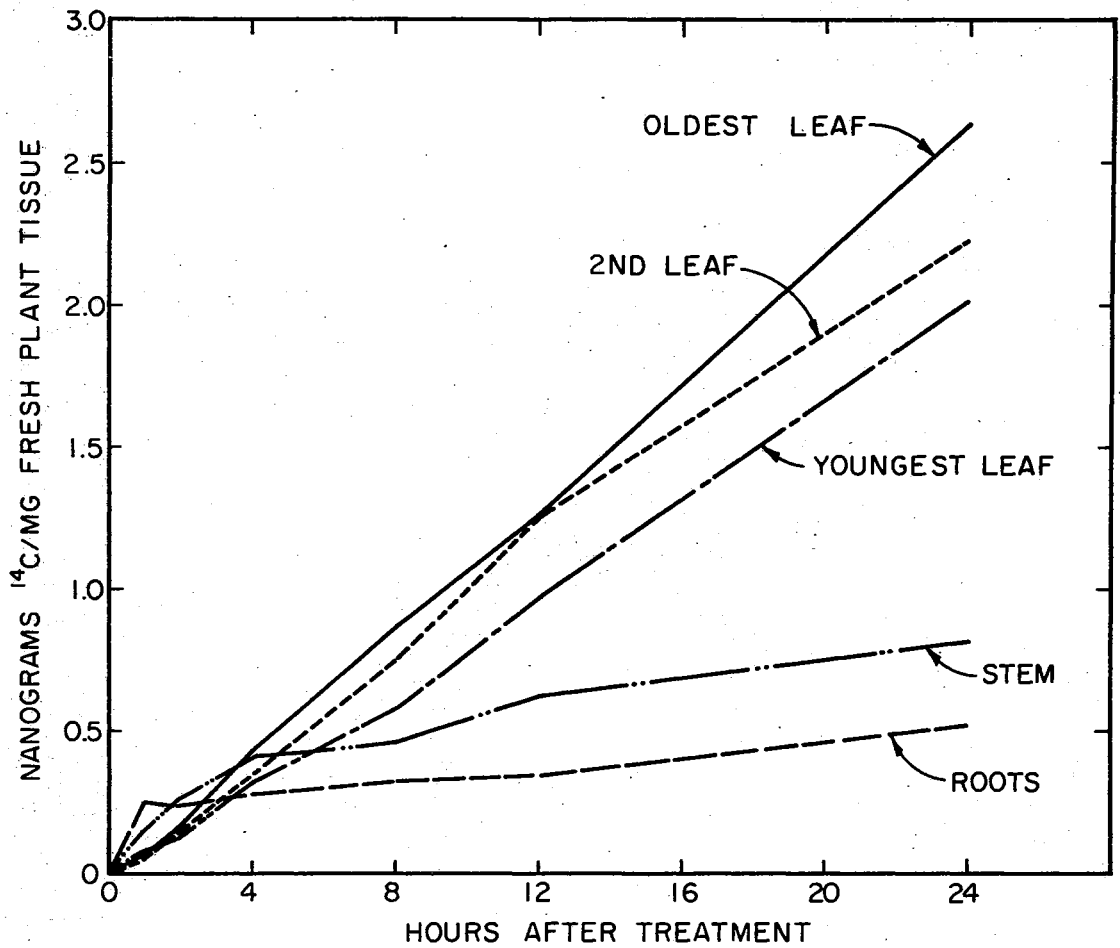


Figure 5. ¹⁴C-Terbutryn Absorption and Translocation in Wheat at Various Time Intervals After Treatment

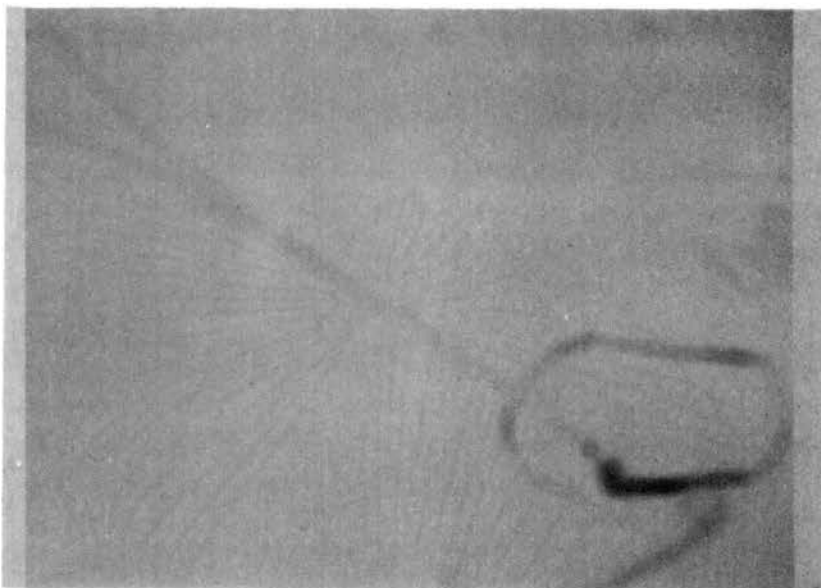


Figure 6. Autoradiogram of Sorghum Plant After 12 Hours Exposure to ^{14}C -Terbutryn Applied Through the Roots.



Figure 7. Autoradiogram of Sorghum Plant After 48 Hours Exposure to ^{14}C -Terbutryn Applied Through the Roots.

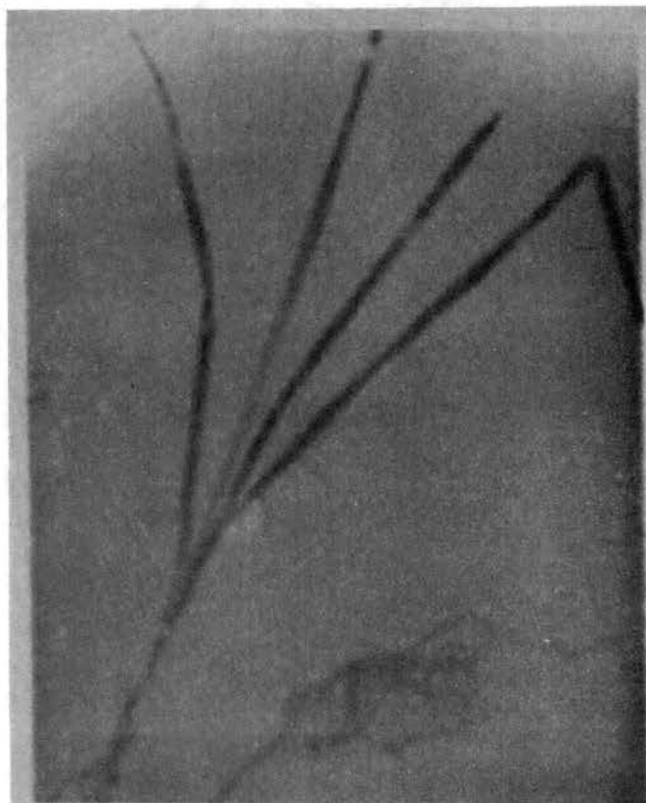


Figure 8. Autoradiogram of Wheat Plant
After 48 Hours Exposure to
 ^{14}C -Terbutryn Applied
Through the Roots.

Labeled Propazine Tracer Studies

The duplicate experiments conducted with labeled propazine were combined into one large experiment. Values for radioactivity are an average of 8 plants per harvest time. The accumulation of radioactivity within each species plant part was expressed as nanograms ^{14}C -herbicide per gram of fresh plant tissue.

Propazine Treatment Time Absorption and Translocation in Sorghum

Sorghum plants were exposed to labeled propazine, harvested at various time intervals, and the absorption and translocation patterns for propazine radioactivity then obtained. At the early harvest periods, propazine radioactivity was negligible in sorghum foliage with more radioactivity in the stem and roots (Table IV and Figure 9). The accumulation of radioactivity in the foliage increased with time of propazine exposure. However, the stem and roots reached a peak in radioactivity accumulation and showed only small increases in radioactivity with time after treatment. The foliage contained more radioactivity than the roots and stem at the final harvest. Older leaves accumulated the most radioactivity of the foliage.

Propazine Treatment Time Absorption and Translocation in Wheat

The absorption and translocation patterns of labeled propazine in wheat were determined. Accumulation of propazine radioactivity was evident in wheat foliage for the early time periods (Table V and

TABLE IV
 ACCUMULATION OF ¹⁴C-PROPAZINE IN SORGHUM PLANT PARTS
 (NANOGRAMS ¹⁴C-HERBICIDE/g FRESH PLANT TISSUE)

Plant Part	Time After Trmt. (Hours)					
	1	2	4	8	12	24
Youngest Leaf	191.62 a*	150.10 b	279.27 b	455.80 a	802.98 a	1316.10 b
Second Leaf	151.67 a	70.15 c	268.23 b	486.66 a	923.88 a	1932.84 a
Oldest Leaf	182.08 a	127.65 b	246.40 b	518.20 a	854.52 a	1846.62 a
Stem	163.22 a	220.35 a	356.12 a	441.90 a	444.65 b	632.33 c
Roots	253.62 a	218.49 a	268.97 b	297.87 b	296.60 c	347.88 d

*Values for each plant part within the temperature range followed by the same letter are not significantly different at the 0.05 level.

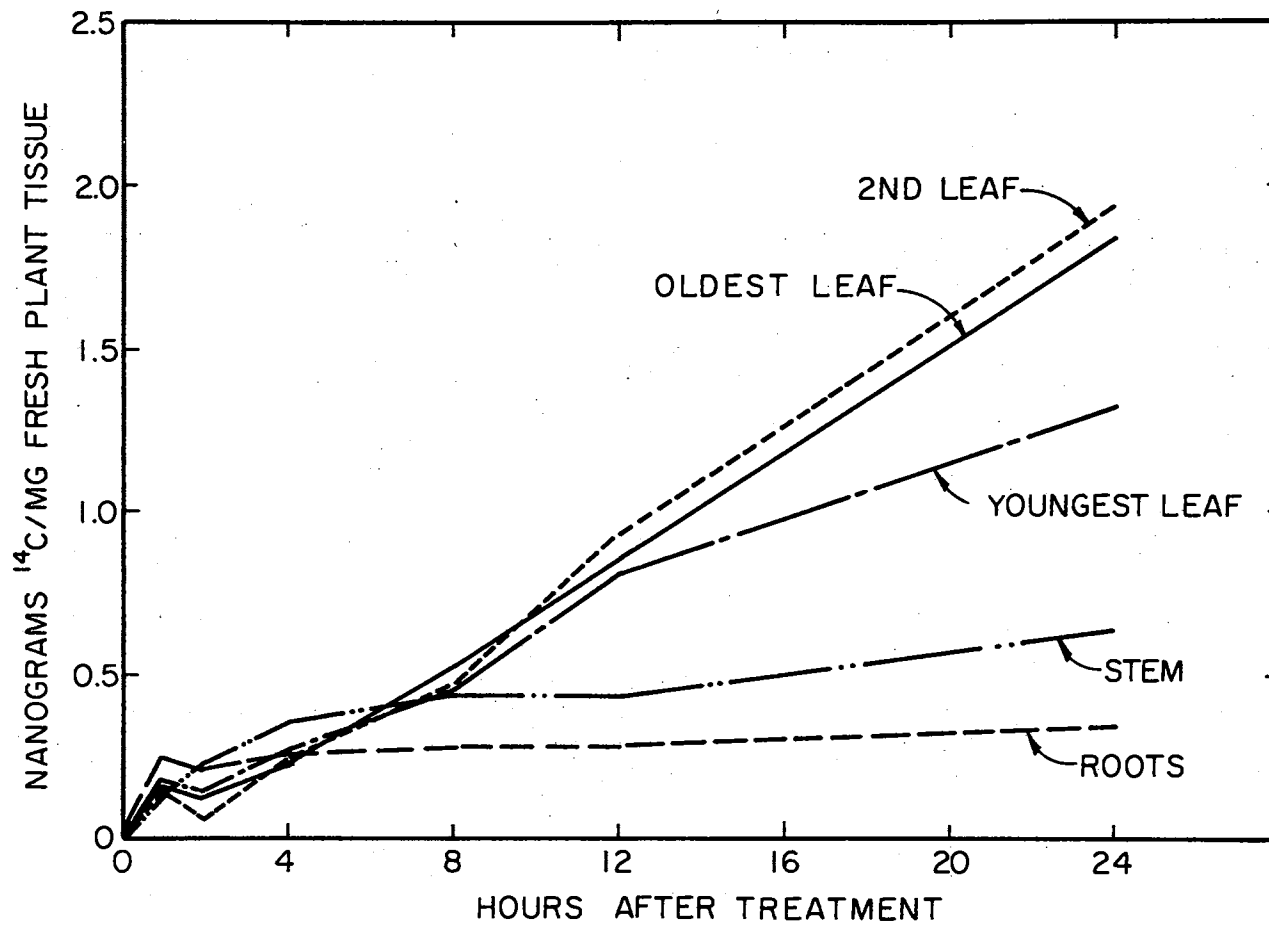


Figure 9. ^{14}C -Propazine Absorption and Translocation in Sorghum at Various Time Intervals after Treatment

TABLE V
 ACCUMULATION OF ¹⁴C-PROPAZINE IN WHEAT PLANT PARTS
 (NANOGRAMS ¹⁴C-HERBICIDE/g FRESH PLANT TISSUE)

Plant Part	Time After Trmt. (Hours)					
	1	2	4	8	12	24
Youngest Leaf	71.40 c*	151.69 c	366.09 c	647.66 b	815.31 b	2183.61 c
Second Leaf	122.14abc	234.46 b	523.80 b	1103.82 a	1219.14 a	2594.56 b
Oldest Leaf	114.83abc	311.26 a	657.44 a	1148.19 a	1350.59 a	3117.26 a
Stem	142.01 ab	158.74 c	216.05 d	235.08 c	299.49 c	327.82 d
Roots	86.48 bc	103.18 c	92.41 e	118.59 c	124.29 c	146.95 d

*Values within each time period followed by the same letter are not significantly different at the 0.05 level.

Figure 10). Root and stem accumulations of radioactivity were small as exposure time increased. At later harvest periods, the foliage was the major site of radioactivity accumulation while the stem and roots contained the least radioactivity. The older leaf contained more labeled propazine than any other plant part.

Temperature Influence on Herbicide Translocation

The influence of different temperatures on herbicidal translocation in wheat and sorghum was investigated by exposing plants to various temperatures. The effect of temperature on herbicidal translocation for each species plant part was analyzed separately. This method was preferable because temperature influence could be more easily observed.

Temperature Influence on Terbutryn

Accumulation in Sorghum

Sorghum plants exposed to different temperatures quantitatively followed different radioactivity accumulation patterns. Plants treated at 32°C accumulated more terbutryn-radioactivity in each plant part than plants treated at 24°C or 16°C (Table VI). Accumulation of radioactivity in the roots appeared not to be influenced by the 24°C and 32°C temperatures but there was significantly less radioactivity accumulation at the 16°C temperature. Roots had the largest amount of radioactivity accumulation of all plant parts for each temperature exposure. Sorghum plants absorbed more radioactivity at the 32°C temperature, with the least radioactivity accumulation at the 16°C temperature.

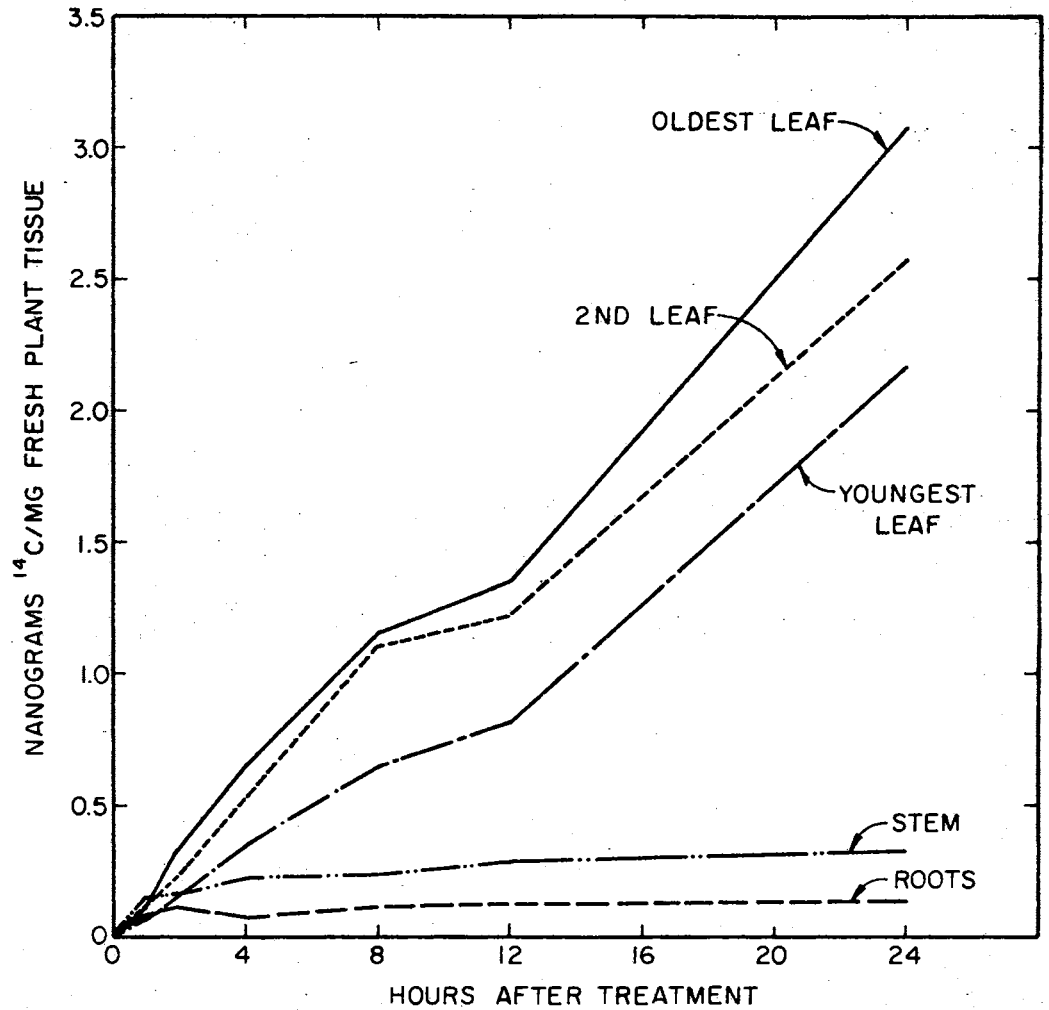


Figure 10. ¹⁴C-Propazine Absorption and Translocation in Wheat at Various Time Intervals after Treatment

TABLE VI
 ACCUMULATION OF ^{14}C -TERBUTRYN IN SORGHUM PLANT PARTS
 UNDER DIFFERENT TEMPERATURES (NANOGRAMS
 ^{14}C -HERBICIDE/g FRESH PLANT TISSUE)

Temp. at Trmt	Plant Parts				
	Youngest Leaf	Second Leaf	Oldest Leaf	Stem	Roots
16°C	151.69 c*	172.85 c	256.90 c	295.22 c	873.49 b
24°C	514.33 b	670.60 b	647.57 b	620.05 b	1713.31 a
32°C	936.31 a	1547.71 a	1547.71 a	907.42 a	1889.73 a

*Values for each plant part within the temperature range followed by the same letter are not significantly different at the 0.05 level.

Temperature Influence on Terbutryn

Accumulation in Wheat

Wheat treated at the highest temperature accumulated more radioactivity in the foliage and stem than the other two temperatures (Table VII). Root accumulation of radioactivity was not different at 24°C and 32°C but was less at the 16°C temperature. Wheat plants absorbed and translocated more radioactivity at the highest temperature (32°C), with the 16°C temperature having the least amount. The major site of radioactivity accumulation proved to be the older leaf for the temperatures evaluated.

Temperature Influence on Propazine

Accumulation in Sorghum

Sorghum plants were treated with labeled propazine and exposed to either 16°C, 24°C, or 32°C during treatment. More translocation and

TABLE VII
 ACCUMULATION OF ^{14}C -TERBUTRYN IN WHEAT PLANT PARTS
 UNDER DIFFERENT TEMPERATURES (NANOGRAMS
 ^{14}C -HERBICIDE/g FRESH PLANT TISSUE)

Temp. at Trmt	Plant Parts				
	Youngest Leaf	Second Leaf	Oldest Leaf	Stem	Roots
16 $^{\circ}$ C	419.70 c*	586.54 c	751.02 c	540.65 c	482.49 b
24 $^{\circ}$ C	1301.60 b	1400.32 b	1787.07 b	739.74 b	580.31 a
32 $^{\circ}$ C	2309.75 a	2714.69 a	3479.74 a	970.51 a	601.67 a

*Values for each plant part within the temperature range followed by the same letter are not significantly different at the 0.05 level.

accumulation of propazine radioactivity in sorghum foliage occurred at the highest temperature (Table VIII). Plants exposed to the lowest temperature had the least radioactivity accumulation. The foliage was the major site for radioactivity accumulation with the roots showing the least at all temperature ranges. Root accumulation of radioactivity appeared to not be influenced by any of the temperatures used.

Temperature Influence on Propazine

Accumulation in Wheat

The affect of increasing temperatures on propazine translocation in wheat is shown in Table IX. More radioactivity was found in all wheat plant parts under the 32 $^{\circ}$ C temperature. The foliage was the major site of radioactivity accumulation with the older leaves containing the most radioactivity. Root radioactivity content was the

TABLE VIII
 ACCUMULATION OF ^{14}C -PROPAZINE IN SORGHUM PLANT PARTS
 UNDER DIFFERENT TEMPERATURES (NANOGRAMS
 ^{14}C -HERBICIDE/g OF FRESH PLANT TISSUE)

Temp. at Trmt	Plant Part				
	Youngest Leaf	Second Leaf	Oldest Leaf	Stem	Roots
16° C	122.06 c*	170.23 c	234.78 c	245.70 b	116.56 a
24° C	341.39 b	516.38 b	395.72 b	295.72 a	166.25 a
32° C	461.82 a	880.18 a	668.43 a	318.94 a	149.87 a

*Values for each plant part within the temperature range followed by the same letter are not significantly different at the 0.05 level.

TABLE IX
 ACCUMULATION OF ^{14}C -PROPAZINE IN WHEAT PLANT PARTS
 UNDER DIFFERENT TEMPERATURES (NANOGRAMS
 ^{14}C -HERBICIDE/g OF FRESH PLANT TISSUE)

Temp. at Trmt	Plant Part				
	Youngest Leaf	Second Leaf	Oldest Leaf	Stem	Roots
16° C	386.51 c*	507.95 c	642.11 c	200.18 b	108.03 ab
24° C	863.31 b	1193.69 b	1351.50 b	234.97 ab	99.24 b
32° C	1524.24 a	2223.04 a	2309.06 a	299.17 a	122.96 a

*Values for each plant part within the temperature range followed by the same letter are not significantly different at the .05 level.

lowest of all plant parts and appeared to not be greatly affected by temperature.

Thin-Layer Chromatography with Sorghum and Wheat

Thin-layer chromatographic procedures were used to determine whether the radioactivity detected in the various plant parts was the parent chemical or a metabolite. The results obtained by liquid scintillation counting of the cut portions from the chromatogram are shown in Tables X and XI. It is believed that at least a part of the terbutryn radioactivity was lost from the chromatogram; possibly by air drying before exposure for the autoradiogram. This was very evident for the sorghum standards because only 60% of the radioactivity could be detected when compared to direct injection into a counting vial followed by liquid scintillation counting. At least a portion of this standard appeared as breakdown products as shown by the autoradiogram. This can be contrasted to wheat where over 95% of the radioactivity from the standards was accounted for by liquid scintillation counting. Since there is a possibility of radioactivity loss, data will be expressed as a percentage of the total radioactivity of the chromatogram found on a certain location on the chromatogram.

Preliminary thin-layer chromatographic procedures using 1 ml of radioactive exposed plant part homogenates proved to be insufficient for visually locating radioactivity spots on the autoradiogram. A volume of 2 ml of plant homogenate was sufficient. However, plant residue plus excessive chlorophyll may have influenced radioactivity mobility.

TABLE X
 CHROMATOGRAPHY OF TERBUTRYN IN SORGHUM
 (NANOGRAMS HERBICIDE)

Characteristic	Stock Terbutryn	Young Leaf	Old Leaf	Roots
Total Radioactive Herbicide Spotted	8620.7	41.7	14.3	107.7
Metabolites	3.9	5.1	1.4	2.5
Parent Chemical	5209.1	1.4	0.7	34.9
Total Radioactive Herbicide Detected After Development	5213.0	6.5	2.1	37.4
<u>Metabolites</u> (%)				
Total Radioactivity Detected	0.07	78.0	66.6	6.7
<u>Parent Chemical</u> (%)				
Total Radioactivity Detected	99.9	22.0	33.4	93.3

TABLE XI
 CHROMATOGRAPHY OF TERBUTRYN IN WHEAT
 (NANOGRAMS HERBICIDE)

Characteristic	Stock Terbutryn	Young Leaf	Old Leaf	Roots
Total Radioactive Herbicide Spotted	7902.5	47.2	47.9	50.3
Metabolites	10.1	2.4	2.2	1.9
Parent Chemical	7534.8	15.8	14.8	9.7
Total Radioactive Herbicide Detected After Development	7544.9	18.2	17.0	11.6
<u>Metabolites</u> (%)				
Total Radioactivity Detected	0.1	13.2	12.9	16.4
<u>Parent Chemical</u> (%)				
Total Radioactivity Detected	99.9	86.8	87.1	83.6

The young leaf, old leaf, and roots were analyzed separately. A 2 ml portion of the plant homogenate was directly injected into a counting vial, counted by liquid scintillation, and used later for a standard of comparison with the radioactivity detected from the cut pieces of the chromatogram. In no case could all the radioactivity be detected indicating that some was probably lost by air exposure or tied up in plant residue. Terbutryn ¹⁴C-radioactivity that was not mobile or did not move with the solvent front was interpreted as break-down products. Sorghum roots had a higher percentage of parent chemical than breakdown products but leaf tissue contained more break-down products (Table X). All plant parts contained at least some breakdown products. The oldest leaf had about the same percentage of parent chemical and metabolites as the youngest leaf. Wheat plant parts contained more parent chemical than metabolites and only slight differences were found among plant parts. Total detectable radioactivity was uniform for all plant parts. Apparently terbutryn was broken down more readily by sorghum than wheat.

Data obtained from the liquid scintillation counting and autoradiography studies help to explain the results obtained from the preliminary bioassay studies in which sorghum was shown to be resistant and wheat susceptible to terbutryn. The absorption, translocation, and accumulative patterns of radioactivity showed the roots of sorghum and the foliage of wheat to be the major sites of accumulation. Accumulation of the herbicide in sorghum roots appears not to be harmful to the plant as indicated by the bioassay studies. Root cells

are probably not as sensitive to the chemical as photosynthetic tissue. Conversely, wheat accumulated more terbutryn-radioactivity in the foliage with a corresponding less amount in the stems and roots. Autoradiography procedures verified the results obtained by liquid scintillation counting. Foliage accumulation of terbutryn was probably toxic to the plant as shown by wheat's low range of tolerance in the bioassay study. Terbutryn acts upon photosynthetic tissue by inhibition of photolysis of water in the photosynthetic process (1). Wheat accumulated more terbutryn-radioactivity in the foliage than sorghum. Thus, the quantitative differences in terbutryn accumulation in the photosynthetic tissue may explain the differences in susceptibility among the two species.

Sorghum and wheat at the later harvest periods accumulated more radioactivity in the older leaves. This indicates apoplastic movement of the herbicide which is a common characteristic of the triazine herbicides. Workers have shown that triazine herbicide movement occurs in the xylem in the transpiration stream (6). The older leaves would contain more well differentiated xylem and non-living tissue than the younger foliage which would help explain part of this pattern of herbicide movement.

The transport of terbutryn to the leaf tissue may or may not be the intact parent chemical in either wheat or sorghum. Thin-layer chromatography showed sorghum contained a higher ratio of terbutryn metabolites in the leaf tissue while wheat foliage contained predominately the intact parent chemical. However, degradation is probably not the major mechanism for selectivity (1). Degradation of terbutryn by plants can occur by oxidation of the methylthio group to

to hydroxy metabolites and by dealkylation of the side chains (1).

Propazine was readily translocated to the foliage of sorghum and wheat with the stems and roots containing the least radioactivity. Wheat foliage contained more radioactivity than sorghum foliage, but the reverse was true of the roots. This can be contrasted to terbutryn where sorghum roots were the major site of herbicide accumulation. Propazine, which is a selective herbicide used in sorghum and is toxic to wheat, may express degradation or some other physiological process rather than differential translocation as its principal means of selectivity. Apoplastic movement of propazine was very dominant as seen by the large accumulation of radioactivity in the older leaf tissue.

Temperature was found to greatly influence the absorption and translocation of both terbutryn and propazine in sorghum and wheat. The high temperature (32°C) promoted the greatest absorption and translocation of radioactivity with the low temperature having the least translocation. One probable reason for this increase of herbicide translocation at the higher temperatures is that the plant is more actively transpiring and the herbicide which is carried in the transpiration stream is translocated quickly and quantitatively within the plant system. Foliage content of radioactivity under the 32°C temperature was very pronounced. However, under the different temperature regimes, sorghum roots were the major site for terbutryn radioactivity while the foliage of wheat contained the most terbutryn and propazine radioactivity.

CHAPTER V

SUMMARY

Growth chamber studies using sorghum and wheat were conducted to gain a better understanding of the mechanism of selectivity of terbutryn. Liquid scintillation counting, autoradiography, and thin-layer chromatography procedures were used to accomplish this purpose. Propazine was used as a basis of comparison in many of the studies.

Preliminary bioassay studies with several grass species showed wheat to be susceptible to terbutryn at herbicide concentrations of 0.5 ppmw; while sorghum's range of tolerance exceeds 4 ppmw. Barnyardgrass and crabgrass were also very sensitive to the chemical. Wheat was chosen as a susceptible and sorghum as a resistant species in the labeled herbicide studies.

Liquid scintillation counting was used to establish the accumulative patterns of terbutryn radioactivity in sorghum and wheat. The accumulation patterns of labeled terbutryn in sorghum proved to be different than wheat. The roots of sorghum and the foliage of wheat were the major sites for radioactivity accumulation when exposed to terbutryn for a period of 24 hours. Autoradiography procedures verified the results obtained by liquid scintillation counting.

Propazine exhibited different patterns of radioactivity accumulation in sorghum than terbutryn-exposed plants. The foliage of wheat and sorghum was the major site for propazine accumulation; however,

wheat absorbed and translocated more propazine radioactivity to the foliage than sorghum.

Apoplastic movement was pronounced for both herbicides in sorghum and wheat. The older leaf tissue contained the most radioactivity implicating xylem as the major tissue for translocation of the herbicides.

Temperature was shown to greatly influence the absorption and translocation of both terbutryn and propazine in sorghum and wheat. The high temperature (32°C) promoted more absorption and translocation of radioactivity than the medium temperature (24°C) with the low temperature in both plant species was very pronounced. In general, root accumulation of radioactivity appeared not to be influenced by temperature. Also, the same accumulative patterns for herbicide radioactivity were observed at all temperatures but the patterns differed quantitatively.

Thin-layer chromatography of terbutryn, using the youngest leaf, oldest leaf, and root homogenates of sorghum and wheat and comparing metabolic products to parent chemical revealed the youngest and oldest leaves of sorghum to have a higher ratio of metabolites than the roots. Wheat had a higher ratio of parent chemical to metabolites than all other plant parts studied.

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