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Selectivity of thiobencarb between two lettuce (*Lactuca sativa, L.*)
cultivars

Reiners, Stephen, Ph.D.
The Ohio State University, 1987
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SELECTIVITY OF THIOBENCARB BETWEEN TWO LETTUCE
(LACTUCA SATIVA, L.) CULTIVARS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Stephen Reiners, B.S M.S.

* * * * *

The Ohio State University
1987

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In Memory of My Father, Herbert C. Reiners
ACKNOWLEDGMENTS

I would like to express my deepest appreciation to my adviser, Dr. Stanley F. Gorski, for his guidance and support during my studies at The Ohio State University. Thanks also to my committee members, Drs. Leo E. Bendixen, Mark A. Bennett, R. Daniel Lineberger, and John C. Peterson for their advice and encouragement.

The technical assistance and friendship of Joe Takayama, Monica Wertz, Gerry Myers, Rich Hassell and Karen Hale is greatly appreciated. To former graduate student Mike Ruizzo, a special thanks for his help and most especially his friendship. For Vicki Gingas and all the other graduate students, thanks for making my years here so enjoyable.

For my brothers, Paul and Michael, and mother Rita, whose love and guidance have been an inspiration in my life, I offer sincere thanks. Finally to my wife, Beth, whose patience, love and encouragement made this work possible, I offer my love and appreciation. Now our life together may really begin.
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CHAPTER I
Mechanisms of Herbicide Selectivity,
A Literature Review

Modern chemical weed control is based on the interspecific selectivity of herbicides among plant families or genera; the ability to control undesirable weed species with little or no effect on the desirable crop. Selectivity is based on many factors, starting with the characteristics of the herbicide formulation, soil and plant retention, environmental influences, and the plant species' growth habit, morphology and physiology (15, 16, 48). Herbicide selectivity between crop and weed leads to the recommendation of a herbicide for use on the entire cultivar spectrum of a species on the assumption that all cultivars will respond similarly (23). This assumption is not, however, always correct. With the advent of the first synthetic herbicides in the 1940's, cases of intraspecific selectivity have been noted (2). The differential response of cultivars within a species forms a useful tool for the researcher. An understanding of the mechanism of selectivity has led to more effective uses of weed killing compounds (22, 38), a better understanding of herbicide
modes of action (4), the incorporation of herbicide tolerant traits into previously susceptible species (13, 31) and a more thorough understanding of basic plant physiological processes.

Selectivity of herbicides may be based on tolerance or resistance. Cultivar tolerance is defined as the ability to withstand herbicide concentrations that cause growth reductions in susceptible species (30). High herbicide rates will eventually affect previously tolerant cultivars. In contrast, herbicide resistance is effective even at the highest rate of herbicides. Herbicide tolerance is relative, resistance is absolute. Examples of differential cultivar tolerance includes soybean (9, 19), tomato (16), and potato (15) cultivars to metribuzin; cabbage cultivars to nitrofen (22, 38), peas to trifluralin (20); tomatoes (34) and pigweed (28) to napropamide; and cucumber (53) to atrazine. The lack of activity of triazine herbicides on Senecio vulgaris biotypes (30) is a classic example of herbicide resistance.

Herbicide selectivity is of particular interest to the vegetable crop grower faced with a more limited herbicide spectrum than agronomic producers. Due to the limited herbicide options, vegetable cultivar susceptibility will usually result in a change to a more tolerant cultivar if available, a more tolerant crop if needed. For lettuce grown on the high organic or muck soils of Ohio, Florida,
Wisconsin and Michigan, this problem is even more severe. Presently, no registered herbicide is available for use. Thiobencarb (S-(4-chlorobenzyl)N,N-diethylthiocarbamate), a thiocarbamate herbicide has been used recently through an emergency use permit. Weed control has been erratic, though differences in cultivar response to the herbicide have been noted in field studies (17). This differential response to thiobencarb leads to a dilemma for growers; rather than using the registered herbicide on all cultivars, cultivars must be selected with acceptable tolerance to the registered herbicide. A more thorough understanding of the mechanism of thiobencarb tolerance may lead to optimizing crop yields for the grower in particular, and add to the information regarding herbicide selectivity in general. To fully understand the mechanism of selectivity, many factors must be considered. Before examining herbicide selectivity, a general review of the mode of action of thiocarbamates will be given.

Thiocarbamate herbicides are a family of weed killing compounds which control the growth of many annual grasses and some selected broadleaves. Though these compounds have been in use for the past thirty years, the primary mechanism of action remains unknown. Possible effects may be seen on cell division (4), elongation (18), pigment production (54), protein synthesis (43), endogenous hormone synthesis (54), and fatty acid synthesis (55). The effect
on fatty acids and lipids has been demonstrated with EPTC and diaallate on spinach (55). Unsaturated fatty acid synthesis was decreased as was the thickness of the cuticle. The composition and thickness of cabbage cuticles were also influenced by EPTC (38).

Thiocarbamates are general meristematic inhibitors. They are applied to the soil and apparently move apoplasticly to the above ground foliage (4). Primarily, these compounds inhibit the above ground growth to a much greater degree than the roots. Leaves may show abnormalities or fasciations which include leaf fusion and cupping and may be arranged in irregular patterns (18). Injury seems to occur at the earliest stages of leaf primordia development in the meristem. Concentration differences at the site of action, or the degree of susceptibility of these tissues may vary by species or within species and account for cases of differential tolerance.

Before a herbicide reaches a plant, many variables exist which influence its phytotoxicity. Differences in soil types, organic matter content, soil tilth, planting depth, soil moisture, cation exchange capacity, light, temperature and rainfall following application may all influence the degree of herbicidal toxicity observed (37). Greater corn injury occurred at 20°C compared to 30°C when treated with EPTC or butylate, both thiocarbamate
herbicides (37). Apparently, the greatest injury occurred when corn coleoptiles were in contact with the herbicide as they emerged from the soil. Environmental conditions favoring emergence, moisture and high temperatures, decreased the period of time in which the coleoptile was in contact with the herbicide. In other studies, planting soybeans deeper, away from the high concentrations of metribuzin near the soil surface, increased tolerance (9). In addition, decreased soybean phytotoxicity was observed as the percentage of soil organic matter increased, likely due to greater adsorption on soil colloids (9).

Phytotoxicity of photosynthetically active compounds such as the triazaines may be related to levels of light reaching plants prior to herbicide treatment (52). One day of 75% shade reduced the tolerance of some tomato cultivars, as well as jimsonweed and velvetleaf, to metribuzin (39, 40). Three days of shade further reduced tolerance. The effect was likely due to a lowered level of storage carbohydrates, making them more susceptible to injury (11). Light may also play an indirect role in the tolerance observed in some cabbage cultivars to nitrofen by influencing cuticle development (38). 'Hybelle', a tolerant cultivar, was found to develop a thicker cuticle than the susceptible 'Rio Verde'. The thicker cuticle led to decreased herbicide retention and consequently, decreased phytotoxicity.
The many variables associated with field studies examining herbicide selectivity make this method problematic. Use of nutrient solution assays in controlled environment chambers eliminates many of the variables. Presently, more than 300 solutions are available for use (47), though the most widely utilized continues to be modified Hoagland's solutions (21). By eliminating the variability of soil assays, and with greater control of moisture, nutrients, light and temperature, the researcher has a better estimate of the absolute level of herbicide tolerance.

In addition to environmental influences on herbicide selectivity, the plant's own morphology may play a role. The composition and thickness of the leaf cuticle continues to play a major role in determining the amount of foliar applied spray remaining on the leaf (42). As reviewed earlier, cuticle thickness determined the degree of cabbage tolerance to nitrofen (38). By physically removing leaf cuticles, formerly tolerant cultivars expressed the same degree of injury. In some cotton cultivars, lysigenous glands present on the leaves accumulated S-triazines and led to a partial tolerance to the herbicide (14). Sequestered within these organs, away from the site of action, the herbicide effects were negligible. Glandless cotton varieties, however, were very susceptible to the herbicide. In addition to specialized organs increasing
tolerance, the growth habit of a species may do the same. Two *Amaranthus* species, *blitoides* S.Wats. and *retroflexus* L., differed in their response to napropamide (28). Examination revealed that the tolerant species had a deeper root system, placing the site of uptake far below the layer of soil where the napropamide concentration was highest. This is a case where the differential tolerance would not be observed utilizing nutrient culture assays only. Even with the many variables associated with soil assays, these may have to be included to completely analyze selectivity mechanisms observed in the field.

An analysis of cultivar growth parameters is often a useful tool in determining the nature of selectivity. Examining the differential response of two cucumber cultivars to atrazine, Werner and Putnam (53) measured leaf area ratios, net assimilation rates and relative growth rates. The susceptible cultivar had significantly greater root biomass and a larger leaf area than the tolerant cultivar. Using radiolabeled atrazine in nutrient solution, the researchers found greater uptake due to the larger root system and a corresponding increase in injury. Smith and Wilkinson (46) found that growth rate differences may account for the tolerance of some tomatoes to metribuzin. To date, little work has been completed examining thiocarbamate tolerance in relation to these factors.
The age of a plant is often critical in determining the degree of injury observed. Most species increase in tolerance as they mature, usually being most susceptible soon after germination, after storage reserves in the seed are utilized but before active photosynthesis (1). Both metribuzin susceptible and tolerant species were shown to be susceptible to the herbicide in the earliest stages of growth (48). Further in development, the susceptible species becomes less sensitive. Similar results have been reported with other herbicides (11, 51).

The most important aspects governing herbicide selectivity are the uptake of the compound, movement to and accumulation at the site of action, and possible metabolism resulting in a decrease in concentration of the toxic molecule. As discussed previously, environmental and morphological factors usually affect selectivity in an indirect way, by altering the amount of herbicide taken up by the plant. The increased uptake of atrazine and chloramben by susceptible cultivars leads to greater phytotoxicity (32, 53). Uptake alone, however, may not be adequate in explaining selectivity differences observed. The researcher needs to know to what extent the herbicide moves to the site of action. Rates of chloramben uptake have been found similar in both tolerant and susceptible cultivars but movement to the site of action, in this case the developing foliage, was limited in tolerant cultivars
More of the chemical remained in the roots with limited herbicidal effects. In sensitive species, greater amounts of herbicide were translocated, essentially overloading the plants' ability to maintain concentrations below a phytotoxic level (10). Uptake and movement of metribuzin to leaf tissue were found similar in two potato cultivars that exhibited selectivity differences (15). Closer examination revealed the radiolabel had moved differently in the leaves of the two cultivars. In the sensitive species, the label was seen throughout the leaf. In the tolerant species, accumulation was limited to the veinal regions, sequestered away from the photosynthetic apparatus of the plant leaf and minimizing plant injury. This study indicates the need to closely examine the site of herbicide accumulation. Studies with thiobencarb comparing susceptible barnyardgrass and tolerant rice, found greater root uptake and foliar translocation in the sensitive species (36). Higher concentrations of parent thiobencarb at the site of action, foliar meristematic tissue, may explain the degree of selectivity observed.

Herbicide metabolism is the single most important aspect governing selectivity. Perhaps the best example occurs with corn and its response to atrazine. Degradation occurs quickly in one of three ways (29); 1) hydroxylation, 2) N-dealkylation of the side chains and 3) conjugation with glutathione. Metabolism lowers the
concentration of herbicide at the site of action and hence, decreases the phytotoxicity. Differential rates of metabolism of EPTC in alfalfa (1); metribuzin in tomatoes (31) and soybeans (46); and chloramben in cucumbers (50) have all been documented. Thiocarbamate metabolism has been most extensively studied with EPTC. In mammalian systems, the parent compound is quickly converted to nontoxic sulfoxides (7). Further degradation involves conjugation with reduced glutathione and the eventual excretion of mercapturic acids (8, 25). The initial step of thiocarbamate metabolism in plants is similar to mammalian systems with the enzyme mediated production of the sulfoxide (5, 24). The sulfoxides are potent carbamoylating agents, to a greater degree than the parent compounds (7). In plants, sulfoxidation is apparently a bioactivation reaction since this is the form with herbicidal activity. Capable of carbamoylating thiol groups, the sulfoxides may affect many growth processes, possibly causing the growth abnormalities observed (33).

The increased potency of the thiocarbamate sulfoxide has been documented with EPTC and pebulate (7). The toxicity is most severe on plants already susceptible to the parent compound and may actually widen selectivity. Apparently, plant species with normally high levels of reduced glutathione and active glutathione S-transferase rapidly metabolize and conjugate the sulfoxide. The result
is the formation of water soluble conjugates with no herbicidal activity. In plants lacking an active pathway, the sulfoxide reaches toxic levels. Hence it may be the rate of metabolism that determines the degree of selectivity between two plant species (41).

In previous metabolism studies with thiobencarb, the sulfoxide was not identified with in vivo mouse systems (27), nor in rice or barnyard millet (35). Rat liver homogenates produced the sulfoxide in vitro (25) and it was found as a soil metabolite (26). Failure to identify the metabolite in plants may be due to a lack of sensitivity in the procedures. One of the unidentified spots on the thin layer plates may have been the sulfoxide. Further metabolism research with thiobencarb should investigate the sulfoxide's role, if any, in determining the mechanism of selectivity.

As demonstrated by Figure 1, many factors influence the selectivity of herbicides (44). The interaction between the herbicide, the environment and the plant all play major roles. An expanded knowledge of herbicide selectivity may lead to optimizing weed control and a more thorough understanding of herbicide modes of action and basic plant physiology.
Figure 1. Factors affecting mechanisms of herbicide selectivity.
HERBICIDE APPLICATION

ENVIRONMENTAL EFFECTS

UPTAKE

TRANSLOCATION

METABOLISM

ACTIVE INTERNAL CONCENTRATION OF TOXIC MOLECULE

SITE(S) OF ACTION

PRIMARY EFFECT

SECONDARY EFFECT

SECONDARY EFFECT

INJURY

TRANSIENT

RECOVERY

IRREVERSIBLE

DEATH
LITERATURE CITED


CHAPTER II

A Nutrient Culture Technique to Measure Lettuce

(Lactuca sativa) Sensitivity to Thiobencarb

ABSTRACT. A new nutrient solution technique was utilized to study lettuce (Lactuca sativa L.) cultivar response to thiobencarb [S-(4-chlorobenzyI) N,N-diethylthiocarbamate]. The nutrient solution provided for fast, reproducible growth of lettuce and may be a useful alternative to the widely used Hoagland's solutions. Modified styrofoam cups with cheesecloth bases provided excellent support for plants and may allow for increased number of treatments in a limited space. Five cultivars were examined for thiobencarb tolerance. 'Great Lakes 366' (tolerant) and 'Dark Green Boston' (susceptible) were identified as having differential levels of tolerance at thiobencarb rates up to 4 uM. Other tested cultivars were intermediate in their response. Differences were observed in dry weight of leaf tissue twelve days after initial treatment. Nutrient culture results agree with assays utilizing muck soils. On the basis of this study, 'Dark Green Boston' and 'Great Lakes 366' appear to be useful cultivars for future studies examining the nature of thiobencarb tolerance.
INTRODUCTION

Chemical weed control is based on the selectivity of herbicides between plant families or genera, though differences in cultivar susceptibility within species have been documented since the introduction of 2,4-D in the 1940's (1). Cultivar tolerance is a useful research tool for studying herbicide mechanisms of action, uptake, and metabolism. Identifying tolerance inheritance traits may be useful for the incorporation of herbicide tolerance into new cultivars (3, 4, 10). Tolerance within horticultural crops is very important due to the limited herbicide options available. Growers, unable to use a registered herbicide on a particular cultivar, must select cultivars for the registered herbicide.

Studies examining cultivar tolerance often utilize nutrient solution assays to measure absolute levels of plant tolerance and eliminate herbicide-soil interactions (6, 16). Currently more than 300 nutrient solutions are available but most research utilizes a modified Hoagland solution, often adapted for a particular plant species (7, 17). Hoagland solution requires exacting accuracy since micronutrients are added individually and may not always
provide the optimum form and levels of nutrients necessary for acceptable plant growth (19).

In addition to the many nutrient solutions available, several methods exist for growing plants within these solutions. Aeration of these solutions may not be necessary if the upper portion of roots remain partially out of solution (7). The ability to support growth of developing plants is an important requirement in any system. A novel method was developed by Machado et al. (11), for examining tomato (**Lycopersicon esculentum** L.) tolerance to metribuzin [4-amino-6-tert-butyl-3-(methyl thio)-as-triazin-(4H)-one]. Styrofoam cups were cut 1 cm from the base and the bottoms discarded. One cm from the first cut, a second cut was made and the styrofoam ring obtained covered with a cheesecloth base and inserted into the modified cup to provide a firm planting grid. Seeds were sown in the cups and floated in large trays filled with herbicide treated nutrient solutions. Growth was uniform and the system provided for easy observation of the developing roots.

Thiocarbamate herbicides are a widely used family of weed control chemicals. Few studies have examined cultivar differences to these compounds as compared to the thorough research conducted on the triazine family of herbicides (3, 4, 8, 9, 11, 15). Thiobencarb is a thiocarbamate herbicide extensively used in rice production and recently for
lettle grown on muck soils. Generally acceptable weed control has been attained though lettuce cultivars varied in response to increasing rates (2, 5). The objectives of this study were: 1) to develop a simple and reproducible nutrient culture bioassay to measure lettuce cultivar response to thiobencarb, and 2) to compare nutrient solution assays to tests utilizing muck soils.
MATERIALS AND METHODS

Nutrient Solution. Eight ounce styrofoam cups were prepared utilizing a modified method of Machado, et al. (11). Prepared eight ounce styrofoam cups with cheesecloth bases were placed in uncut cups filled with 60 mls of 75% nutrient solution. Nutrient solution was adapted from the Nutrient Film Technique of Vetanovetz and Peterson (19). Each liter contained 910 mg Peter's Hydrosol\(^1\), 24 mg S. T.E.M.\(^1\) (Soluble Trace Element Mix), 740 mg KNO\(_3\), 1.47 g CaN0\(_3\), 190 mg MgSO\(_4\), and 39 mg Sequestrene 330\(^2\). KOH was added to adjust the pH to 6.5 and 1 mg/l of copper in the form of CuSO\(_4\) was added to control algal growth. Five lettuce cultivars, 'Great Lakes 366', 'Dark Green Boston', 'Valmaine', 'Bibb', and 'Slobolt' were seeded on the cheesecloth bases and placed in a growth chamber at 20\(^\circ\) C, 70% + 10% relative humidity, 14:10 h light period and a mean Photosynthetic Photon Flux Density (PPFD) of 300 to 400 umol m\(^{-2}\) s\(^{-1}\). Four days after seeding, four uniform seedlings were selected from each

---

\(^1\)Peters Fertilizer Products, W. R. Grace and Co., Fogelsville, PA 18051

\(^2\)Agriculture Division, CIBA-GEIGY Corp., Greensboro, NC 27409.
cultivar and placed in 55ml of 75% nutrient solution containing technical grade thiobencarb at rates of 0, 2.0, 4.0 and 6.0 uM. Technical grade herbicide (95% purity) was solubilized in a 95% methanol and octoxynol (9 POE) (polyoxyethylene octyl phenyl) surfactant (95:5 v/v). Solubilized herbicide was added to nutrient solutions producing a stable emulsion. Solvent concentration did not exceed 0.1% and this was found to have no effect on plant growth. A second study was conducted similar to the first except only 'Great Lakes 366' and 'Dark Green Boston' were used and thiobencarb rates ranged from 0 to 6.0 uM in 1.0 uM increments. Solutions were changed every four days to maintain herbicide and nutrient concentrations. Twelve days after the original treatment, plants were harvested, roots and leaves dried at 70°C for 48h and dry weights recorded. Lettuce injury was evaluated throughout the study. I50 values, the herbicide concentration necessary to inhibit dry weight by 50%, were calculated. Differences in I50 values between cultivars were used to estimate the level of tolerance (3). Treatments were replicated four times, with four plants in one cup representing one replication. Treatments were completely randomized and experiments were repeated twice in time.

3Triton X-100; Rohm and Haas Co., Independence Mall West, Philadelphia, PA 19105.
Soil assay. Studies were conducted to compare a muck soil assay to the nutrient culture results. A Carlisle muck containing 75% organic matter with a pH of 5.3 was placed in greenhouse flats (35x25x6cm). 'Great Lakes 366' and 'Dark Green Boston' were uniformly seeded in alternate rows with three rows of each cultivar per flat. After seeding flats were sprayed with technical grade thiobencarb, prepared as described previously, at rates of 0 to 13.5 kg ai/ha in 2.24 kg/ha increments. Application was made with a conveyor-type greenhouse sprayer at a pressure of 207 kPa and 470 l/ha. Herbicide was incorporated with 1.25 cm of water applied as a mist and flats were placed in growth chambers with the conditions described previously and watered as needed. Sixteen days after seeding, top dry weights were taken from 20 cm of row. Treatments were replicated three times and the entire study replicated twice in time.

Statistical Analysis. Data from replications in time were combined, subjected to ANOVA and least square polynomial fitting and presented as % of control. Means were separated at the 5% level of significance using Fisher's protected LSD.
RESULTS AND DISCUSSION

Nutrient Solution. Utilizing nutrient solutions to screen plant populations for herbicide tolerance is a valuable method for eliminating herbicide-soil interactions. The hydroponic solution adapted from Vetanovetz and Peterson (19), provided consistent and reproducible lettuce growth. This solution was also used in varying strengths in studies with corn and cucumbers and provided an excellent medium for growth. Placing a modified styrofoam cup within an uncut one rather than floating the cups in large reservoirs is an advantage. It allows for larger numbers of treatments in limited growth chamber space and allows for greater randomization in the experimental design. It also permits for aeration of the upper roots which is necessary for good growth. The limited reservoir of 55 mls in the cup requires that levels of nutrients be at a maximal concentration. The modified cup technique is a useful tool for both research and teaching while the nutrient solution provides for excellent growth.

Adding chelated micronutrients in already prepared soluble fertilizers (Peter's Hydrosol and S.T.E.M.), eliminates the need for addition of individual salts to the solution. Hence, this NFT solution is easier to prepare than Hoaglands solution and may lessen the possibility of errors associated with the addition of micronutrients.

The five lettuce cultivars exhibited significant reductions in leaf dry weights as thiobencarb rates increased (Figure 2). The cultivar response varied at rates below 4.0 uM with 'Great Lakes 366' being the most tolerant and 'Dark Green Boston' the most susceptible. 'Slobolt', 'Valmaine' and 'Bibb' expressed intermediate levels of tolerance. The greater reduction in yield with 'Dark Green Boston' agrees with earlier field work demonstrating yield reductions at rates greater than 6.7 kg/ha (5). Symptoms of herbicide injury first appear as abnormal growth of secondary leaves resulting in stunting as early as four days after treatment. By twelve days after treatment, growth inhibitions of leaf tissue were significantly greater in 'Dark Green Boston' than 'Great Lakes 366' in the 2.0 uM treatment. The inhibition of root growth seemed to be a secondary effect due to the decrease in leaf growth. The primary effect on above ground growth agrees with the mode of action of other thiocarbamates (12). High rates of thiobencarb resulted in a general inhibition of secondary leaf growth in all cultivars.
Figure 2. Leaf dry weight of 'Great Lakes 366' (GLA), 'Slobolt' (SLB), 'Summer Bibb' (BIB), 'Valmaine' (VLM), and 'Dark Green Boston' (BOS) lettuce cultivars grown in nutrient solution and treated with thiobencarb at rates of 0, 2, 4, and 6 uM. Data is presented as percent of untreated control.
Figure 2.
On the basis of the first study, 'Great Lakes 366' and 'Dark Green Boston' were selected as the two cultivars exhibiting the greatest differences in tolerance to the herbicide. The number of thiobencarb treatments were increased to better study the levels of cultivar tolerance and determine the $I_{50}$ values. Thiobencarb concentrations below 4.0 $\mu$M resulted in significant cultivar differences (Figure 3). Leaf growth of 'Dark Green Boston' was inhibited 50% at 3.0 $\mu$M while 'Great Lakes 366' required a concentration of approximately 5.0 $\mu$M to attain the same reduction. The $I_{50}$ value of 'Great Lakes 366' is more than 65% higher than that of 'Dark Green Boston'.

No significant decrease in growth occurred in 'Great Lakes 366' even at the highest rate tested on the muck soil (Figure 4). The growth reduction of 'Dark Green Boston' is significant, with greater than a 50% reduction occurring at the highest level. Phytotoxic symptoms appeared as a decrease in plant population, stunting and leaf fusion, similar to the nutrient solution studies. Results of this study indicate that the tolerance of 'Great Lakes 366' is not solely related to the method of treatment, since tolerance was observed in two systems, nutrient solution and soil.

The hydroponic solution used in Nutrient Film Techniques is adaptable to plant culture on a much greater scale and may provide an alternative to the many
Figure 3. Leaf dry weight of 'Great Lake 366' (GLA) and 'Dark Green Boston' (BOS) grown in nutrient solution and expressed as percent of untreated control. Bars represent the LSD at the 5% level of significance.
Figure 3.

LEAF DRY WEIGHT (% of control)

THIOBENCARB CONCENTRATION (uM)

GLA \( r^2 = .99 \) \( y = 99.67 + 20.52x - 49.83x^2 \)

BO3 \( r^2 = .92 \) \( y = 97.42 - 73.65x + 16.19x^2 \)
Figure 4. Leaf dry weight of 'Great Lake 366' (GLA) and 'Dark Green Boston' (BOS) grown in muck soil and expressed as percent of untreated control. Bars represent the LSD at the 5% level of significance.
Figure 4.
modified Hoagland solutions currently utilized. 'Dark Green Boston' (susceptible) and 'Great Lakes 366' (tolerant) were significantly different in their response to thiobencarb in nutrient solution and soil assays. These two cultivars may prove useful in future studies determining the nature of thiobencarb tolerance.
LITERATURE CITED


13. Parker, C. Factors affecting the selectivity of 2,3-dichloroallyl diisopropylthiophosphorocarbamate (Di-allate) against *Avena* spp. in wheat and barley. *Weed Res.* 3:259-76.


Chapter III

Uptake, Translocation and Metabolism of Thiobencarb in Two Lettuce (Lactuca sativa) Cultivars

ABSTRACT. Two lettuce cultivars exhibiting differential levels of tolerance to thiobencarb in soil and nutrient solution assays were examined. Seedlings of 'Dark Green Boston' (BOS), a susceptible cultivar, were found to show significant inhibitions in foliar growth as compared to the tolerant 'Great Lakes 366' (GLA). Growth reductions occurred at rates of 3μM thiobencarb as soon as four days after treatment. In addition, growth abnormalities including fused leaves were observed, indicating inhibition early in leaf development at the meristem. Greater amounts of $^{14}$C-thiobencarb were absorbed from nutrient solution by BOS, probably due to a significantly greater root system at the time of treatment. This greater uptake and accumulation of $^{14}$C-label in the leaves, as well as significantly greater amounts of parent $^{14}$C-thiobencarb in the foliage of BOS may account for the selectivity observed. Metabolism of $^{14}$C-thiobencarb occurred rapidly in both cultivars, with the apparent production of herbicide conjugates accounting for more than 90% of the
extracted radiolabel by the end of the study. A thiobencarb sulfoxide metabolite was not identified in these studies. This indicates the metabolism of thiobencarb differs from other members of the thiocarbamate family of herbicides.
INTRODUCTION

Thiobencarb [S-(4-chlorobenzyl)N,N-diethylthiocarbamate] is a thiacarbamate herbicide widely used in rice production. It controls many annual grasses and some selected broadleaves, with good selectivity between rice and weeds. Recently, thiobencarb has been tested as a herbicide replacement for CDEC (2-chloroallyl diethylthiocarbamate) on lettuce grown on muck soils in Ohio, Florida and Wisconsin (3, 8). Though weed control was variable, crop response varied by cultivar. Nutrient solution assays determined two species with a wide tolerance difference. 'Dark Green Boston' (BOS) (susceptible) and 'Great Lakes 366' (GLA) (tolerant), were significantly different at concentrations up to 4.0 uM. Phytotoxicity symptoms include reduced foliar growth, and cupped, fused and rolled leaves, abnormalities normally associated with this family of herbicides (9).

Cases of intraspecific selectivity with thiacarbamates have not been studied as extensively as with other herbicide families. Cultivar differences amongst the triazine herbicides have been related to differential uptake, movement (22), accumulation (20), metabolism (1) and anatomical differences within the chloroplast thylakoid
membrane (2). In examining differences between rice (tolerant) and barnyardgrass (susceptible) response to thiobencarb, Nakamura, et al (15) found greater root uptake and translocation within the susceptible species. These differences may result in the selectivity observed.

The metabolism of thiacarbamates has been studied most extensively with EPTC (S-ethylidipropylthiocarbamate). In mammalian systems, the parent compounds are quickly converted to non-toxic sulfoxides (6) and eventually conjugated with reduced glutathione (4, 10). In plants, the initial step is similar with the enzyme mediated production of the sulfoxide (4). The EPTC sulfoxide, however, is a strong carbamoylating agent, much greater than the parent compound. It is the sulfoxide that apparently has the herbicidal activity (6). Differences in the rate of sulfoxidation and its eventual degradation may account for the selectivity of EPTC (18). Thiobencarb sulfoxides have been observed in animal systems (12) and as a soil metabolite (11), but not as an identified metabolite in rice or barnyard millet (14). The presence of this metabolite in particular and the overall uptake, movement and metabolism in general, may account for the observed selectivity in lettuce.

The objectives of this study were: (1) to compare and contrast the growth of susceptible and tolerant lettuce cultivars to thiobencarb applied to the roots; (2) to
define the role of uptake and translocation of $^{14}$C-thiobencarb in determining selectivity; and (3) to characterize, quantify and contrast the metabolism of $^{14}$C-thiobencarb in two lettuce cultivars.
MATERIALS AND METHODS

General Conditions. Eight ounce styrofoam cups were prepared utilizing the method described by Machado, et al. (13). Seeds of BOS and GLA were sown on cheesecloth bases in 60 mls of 75% nutrient solution (21) and placed in a controlled environment chamber. Conditions were 20°C ± 2°C, 70% ± 10% relative humidity, 14:10h photoperiod and a photosynthetic photon flux density of 300 to 400 umol m⁻² s⁻¹ at the leaf surface. Four days after seeding, four uniform seedlings were selected and placed in 55 mls of 75% nutrient solution containing either technical grade or ¹⁴C-thiobencarb. Herbicide was solubilized in 95% methanol and octoxynol (9 POE) (polyoxyethylene octyl phenyl)¹ (95:5, v/v). Solvent did not exceed 0.1% and was found to have no effect on plant growth. Solutions were changed every four days to maintain herbicide and nutrient concentration. Four plants from each cup were combined as a single replication to provide enough biomass for analysis.

¹Trigon X-100, Rohm and Haas Co., Independence Mall West, Philadelphia, PA 19105.
Growth Study Assay. Four days after seeding, four uniform seedlings were selected and placed in a 3uM technical grade thiobencarb nutrient solution. At 0, 4, 8 and 12 days after the original treatment, plants were harvested and divided into leaf and root samples by cutting 2 mm below the cotyledons. Tissue was dried at 70°C for 48 hours and dry weights recorded. Lettuce injury was visually observed throughout the study. Treatments were replicated four times and the experiments repeated twice in time. Data from both experiments were combined.

Uptake and Translocation. Uniformly ring labeled $^{14}$C-thiobencarb (4 mCi mmole$^{-1}$) was received as a liquid with a radiolabel purity found to be greater than 99%. The herbicide was solubilized as described previously and 0.51 uCi was added to each cup for a final herbicide concentration of 3uM. Lettuce plants were harvested 1, 4, and 12 days after the original treatment. Adsorbed herbicide was removed from the roots by immersion in 10 ml of 10% aqueous acetone and shaken for 30s. One ml aliquots from the remaining nutrient solution and root wash were added to Ready-Solv EP$^2$ liquid scintillation cocktail for quantification. This was considered to be unabsorbed herbicide.

Plants were divided into root and leaf samples, quickly frozen in liquid N<sub>2</sub> and lyophilized. The amount of radioactivity was quantified by combustion<sup>3</sup> and liquid scintillation spectrometry. Counts per minute were corrected for combustion efficiency and background. Quench correction was achieved by internal standardization and data converted to disintegrations per minute. Four replications were established in this study with one being used for autoradiography. The method of Crafts and Yamaguchi (7) was utilized. Exposure was for a period of four days after which time, films were developed.

**Metabolism.** Plants were grown as described for the absorption, translocation study with the exception being that 0.31 uCi of 14C-thiobencarb was added to each treatment. Following lyophilization, roots and leaves were homogenized separately in 90% methanol for 30s. The homogenate was centrifuged, supernatant removed and residue reextracted. Supernatants were combined and dried under nitrogen. The dried remainder was resuspended in methanol and sampled for the radiolabel by liquid scintillation counting (LSC). Two to 4 ul aliquots were spotted on precoated silica gel thin layer chromatography (TLC) plates (0.25 mm)<sup>4</sup>. Plates

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<sup>3</sup>Packard Tricarb Model 306. Packard Instruments, Downers Grove, IL.

<sup>4</sup>Silica gel F-254 plate. Sigma Chemical Co., St. Louis MO.
were developed in one of two solvent systems: I. Toluene, Acetone, Acetic Acid (60:1.5:1.5, v/v/v); and II. Benzene, Ethanol, Acetic Acid (60:0.5:4, v/v/v), to a height of 10 cm. Labeled areas were determined by autoradiography and R_f values determined and compared to known standards for identification. Radioactive areas were scraped from the plate and eluted with 1 ml methanol, for one minute. Liquid scintillation cocktail was added and radioactivity quantified by LSC. Data is presented as the percentage of total radioactivity spotted. This experiment was replicated four times.

Statistical Analysis. Treatments were completely randomized within the growth chamber. All data were subjected to ANOVA and means separated at the 5% level of significance using Fisher's protected LSD.
RESULTS AND DISCUSSION

Growth Study Assay. From previous work, BOS lettuce was found susceptible while a crisphead type GLA was found tolerant to rates of thiobencarb up to 4.0 μM in nutrient solution. Significant differences exist between the untreated controls of the cultivars in terms of both root and leaf biomass at the time of treatment (Day 0), when plants are four days old (Tables 1 and 2). BOS had significantly greater foliage (leaf and stem) and an almost 50% greater root system than GLA in terms of dry weight. The early differences between the untreated controls of BOS and GLA is not statistically evident by day four, though a trend towards a larger root system in BOS continues, becoming statistically significant again by day 12. Differences were evident by the fourth day after treatment in the developing secondary leaf tissue of the treated BOS. No differences existed in the root systems of either of the cultivars. The greatest reduction is in the foliage of treated BOS as compared to the untreated control, which by day 12 is reduced 57%. No significant differences in leaf or root dry weights existed at any time between treated GLA and untreated controls. The decline in root biomass of treated BOS as compared to the control at
Table 1. Effect of thiobencarb on the foliar dry weights (mg) of 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) lettuce over time.\textsuperscript{a}

<table>
<thead>
<tr>
<th>RATE ( (\mu M) )</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.23</td>
<td>12.58</td>
<td>54.53</td>
<td>175.20</td>
</tr>
<tr>
<td>3.0</td>
<td>--</td>
<td>11.01</td>
<td>42.04</td>
<td>100.76</td>
</tr>
<tr>
<td>LSD</td>
<td>0.92</td>
<td>2.03</td>
<td>8.95</td>
<td>22.3</td>
</tr>
</tbody>
</table>

\( \text{LSD} \)'s are calculated at the 5% level of significance.
Table 2. Effect of thiobencarb on the root dry weights (mg) of 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) lettuce over time.\textsuperscript{a}

<table>
<thead>
<tr>
<th>RATE (uM)</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOS</td>
<td>GLA</td>
<td>LSD</td>
<td>BOS</td>
</tr>
<tr>
<td>0</td>
<td>1.53</td>
<td>1.04</td>
<td>0.16</td>
<td>4.84</td>
</tr>
<tr>
<td>3.0</td>
<td>--</td>
<td>--</td>
<td>4.56</td>
<td>4.21</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\textsuperscript{a}LSD's are calculated at the 5% level of significance
day 12 may well be a secondary response due to a decline in available photosynthetic area (16).

The expected site of activity for thiocarbamate herbicides is the above ground meristematic tissue (2). This is the case with thiobencarb since the developing leaf tissue of the susceptible species is inhibited four days after treatment. Observed abnormalities became evident between day 4 and 8 as secondary leaves emerged stunted and sometimes fused. This fusion of developing leaves was seen both in nutrient solution and in earlier field studies (Figure 5). Observed growth abnormalities are consistent with symptoms of other thio and dithiocarbamate herbicides in lettuce and pine (9). Morphological studies of developing barnyardgrass and bearded sprangletop treated with thiobencarb resulted in severe irregularities of leaves while still within the coleoptile and kinking of the first internodal region (19). Once leaf abnormalities were observed in these species, removal from herbicide would not result in any recovery of already misshapen leaves. New leaves, however, would emerge normally and show no symptoms. This indicates the effect may be occurring early in leaf primordia development. Herbicide accumulation at this site, the meristem, is characteristic of this family of chemicals, and may result in the lettuce injury observed.
Figure 5. Leaf abnormalities of 'Dark Green Boston' lettuce treated with thiobencarb.
Absorption and Translocation. Significantly greater amounts of $^{14}\text{C}$-thiobencarb was absorbed and accumulated in BOS compared to GLA by four days after treatment (Table 3). This difference was maintained even at day 12. Similar results are observed in which the percentage of applied label within plants was greater in BOS, with close to 50% of the applied label within this cultivar at day twelve (Table 4). Previous work demonstrated BOS to have a significantly greater root system at the time of treatment. This greater root system may have been responsible for the greater uptake of $^{14}\text{C}$-thiobencarb from the nutrient solution.

Since the primary site of activity is the developing leaves of susceptible plants, concentrations of thiobencarb in this tissue may be critical for the development of phytotoxicity. The concentration of label is consistently greater in the roots of both cultivars with a greater concentration found in the tolerant cultivar 12 days after treatment (Table 3). Since root inhibition is secondary, higher concentrations here would not necessarily be more phytotoxic. The accumulation of $^{14}\text{C}$-compounds in the root may actually increase a species' tolerance to a chemical with its primary activity in the above ground portion (17). Significant differences were observed in the concentration of label in the leaves at four days after treatment. Thiobencarb concentrations in BOS were twice
Table 3. Total uptake and concentration of $^{14}$C-thiobencarb in 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) lettuce over time.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Days After Treatment</th>
<th>Cultivar</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Uptake (DPM's)</td>
<td>BOS</td>
<td>65031</td>
<td>333712</td>
<td>1599061</td>
</tr>
<tr>
<td></td>
<td>GLA</td>
<td>62011</td>
<td>251418</td>
<td>1349371</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>78793</td>
<td>245315</td>
<td></td>
</tr>
<tr>
<td>DPM's/mg d.w. of leaves</td>
<td>BOS</td>
<td>4684</td>
<td>13998</td>
<td>1913</td>
</tr>
<tr>
<td></td>
<td>GLA</td>
<td>7066</td>
<td>6076</td>
<td>1606</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>4703</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>DPM's/mg d.w. of roots</td>
<td>BOS</td>
<td>31352</td>
<td>48821</td>
<td>40139</td>
</tr>
<tr>
<td></td>
<td>GLA</td>
<td>30995</td>
<td>51281</td>
<td>53396</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>NS</td>
<td>6100</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} LSD's are calculated at the 5\% level of significance.
Table 4. Distribution of applied $^{14}$C-thiobencarb in 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) lettuce plants over time.$^a$

<table>
<thead>
<tr>
<th>Assayed $^{14}$C Cultivar</th>
<th>Days after treatment</th>
<th>Days after treatment</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of control</td>
<td>% of control</td>
<td>% of control</td>
</tr>
<tr>
<td></td>
<td>DAY 1</td>
<td>DAY 4</td>
<td>DAY 12</td>
</tr>
<tr>
<td>Intact plant</td>
<td>BOS</td>
<td>5.73</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>NS</td>
<td>6.8</td>
</tr>
<tr>
<td>Root Wash</td>
<td>BOS</td>
<td>0.22</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Nutrient</td>
<td>BOS</td>
<td>84.20</td>
<td>54.60</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>NS</td>
<td>5.9</td>
</tr>
<tr>
<td>Unrecovered$^b$</td>
<td>BOS</td>
<td>9.85</td>
<td>15.41</td>
</tr>
</tbody>
</table>

$^a$ LSD's are calculated at the 5% level of significance

$^b$ Unrecovered = $^{14}$C not accounted for at each exposure period
that found in GLA. It is interesting to note that the phytotoxic symptoms are observed soon after this accumulation in the leaves of the sensitive cultivar.

Autoradiography further supports the conclusion that the primary accumulation of $^{14}\text{C}$-label is in the roots (Figures 6 and 7). Greatest accumulation in the above ground tissue is in the area of the meristem and cotyledons, with limited movement into the secondary leaves of both cultivars. The lack of movement into secondary leaves accounts for the decrease in label concentration observed in the leaf tissue between day 4 and 12. The greater accumulation of $^{14}\text{C}$-label in the developing leaves of BOS may account for the increased sensitivity of this cultivar.

Metabolism. As rapidly as one day after the roots are exposed to $^{14}\text{C}$-thiobencarb in nutrient solution, approximately one half remains as the parent compound within the roots of either cultivar (Tables 5 and 6). A trend towards greater concentrations of unmetabolized $^{14}\text{C}$-thiobencarb in the roots of BOS may lead to the greater movement of the parent molecule to the leaves of this cultivar. Significantly greater amounts of $^{14}\text{C}$-thiobencarb were found in the leaves of this cultivar (Tables 7 and 8) as compared to GLA. A metabolite with $R_f$ values just above thiobencarb ($R_f=0.64$, 0.68) in both solvent systems was tentatively identified as
Figure 6. 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) lettuce plants 1, 4, and 12 days after treatment with $^{14}$C-thiobencarb in nutrient solution.
Figure 7. Translocation and distribution of $^{14} \text{C}$-thiobencarb as demonstrated by autoradiography in 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) lettuce, 1, 4, and 12 days after treatment.
Table 5. Distribution of $^{14}$C-thiobencarb and $^{14}$C-metabolites extracted from 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) roots and separated by solvent system I.\textsubscript{ab}

<table>
<thead>
<tr>
<th>Rf</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOS</td>
<td>GLA</td>
<td>LSD</td>
</tr>
<tr>
<td>0</td>
<td>36.6</td>
<td>49.1</td>
<td>NS</td>
</tr>
<tr>
<td>0.6 \textsuperscript{d}</td>
<td>56.3</td>
<td>41.5</td>
<td>NS</td>
</tr>
<tr>
<td>0.64</td>
<td>5.7</td>
<td>6.5</td>
<td>NS</td>
</tr>
<tr>
<td>0.85</td>
<td>2.0</td>
<td>0.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Toluene, Acetone, Acetic Acid (60:1.5:1.5, v/v/v).

\textsuperscript{b} LSD's are calculated at the 5% level of significance.

\textsuperscript{c} Percent of total $^{14}$C in each aliquot spotted on plate

\textsuperscript{d} $^{14}$C-thiobencarb
Table 6. Distribution of $^{14}$C-thiobencarb and $^{14}$C-metabolites extracted from 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) roots and separated by solvent system II.\(^{ab}\)

<table>
<thead>
<tr>
<th>$R_f$</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOS</td>
<td>GLA</td>
<td>LSD</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>36.2</td>
<td>47.4</td>
<td>NS</td>
</tr>
<tr>
<td>0.68d</td>
<td>57.9</td>
<td>44.6</td>
<td>NS</td>
</tr>
<tr>
<td>0.75</td>
<td>4.6</td>
<td>6.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Benzene, Ethanol, Acetic Acid (60:0.5:4, v/v/v).
b LSD's are calculated at the 5% level of significance.
c Percent of total $^{14}$C in each aliquot spotted on plate.
d $^{14}$C-thiobencarb.
Table 7. Distribution of $^{14}\text{C}$-thiobencarb and $^{14}\text{C}$-metabolites extracted from 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) foliage and separated by solvent system

<table>
<thead>
<tr>
<th>Rf</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOS  GLA LSD</td>
<td>BOS  GLA LSD</td>
<td>BOS  GLA LSD</td>
</tr>
<tr>
<td>0</td>
<td>65.6 74.9 7.0</td>
<td>97.2 96.6 NS</td>
<td>94.5 100.0 0.5</td>
</tr>
<tr>
<td>0.6d</td>
<td>27.8 19.0 4.7</td>
<td>1.6 1.5 NS</td>
<td>3.4 0.0 0.3</td>
</tr>
<tr>
<td>0.64</td>
<td>- - -</td>
<td>0.0 0.6 NS</td>
<td>1.4 0.0 0.2</td>
</tr>
</tbody>
</table>

a Toluene, Acetone, Acetic Acid (60:1.5:1.5, v/v/v).
b LSD's are calculated at the 5% level of significance.
c Percent of total $^{14}\text{C}$ in each aliquot spotted on plate.
d $^{14}\text{C}$-thiobencarb
Table 8. Distribution of $^{14}$C-thiobencarb and $^{14}$C-metabolites extracted from 'Dark Green Boston' (BOS) and 'Great Lakes 366' (GLA) foliage and separated by solvent system II.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>R$_f$</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BOS</td>
<td>GLA</td>
<td>LSD</td>
</tr>
<tr>
<td>0</td>
<td>63.0</td>
<td>78.0</td>
<td>3.8</td>
</tr>
<tr>
<td>0.06</td>
<td>4.1</td>
<td>3.0</td>
<td>NS</td>
</tr>
<tr>
<td>0.68\textsuperscript{d}</td>
<td>31.8</td>
<td>18.8</td>
<td>4.5</td>
</tr>
<tr>
<td>0.75</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Benzene, Ethanol, Acetic Acid (60:0.5:4, v/v/v).

\textsuperscript{b} LSD's are calculated at the 5% level of significance.

\textsuperscript{c} Percent of total $^{14}$C in each aliquot spotted on plate

\textsuperscript{d} $^{14}$C-thiobencarb
4-chlorobenzaldehyde by comparison with analytical standards. This compound was consistently found in greater amounts in the roots of both cultivars. Though 4-chlorobenzylaldehyde was not identified in previous metabolic studies in rice or barnyard millet (14), it has been identified in soil degradation studies (11). This metabolite and any others occurring would seem to be metabolic intermediates in the formation of herbicide conjugates most likely found at the origin of both solvent systems (14).

Four days after treatment began, significantly greater amounts of unmetabolized thiobencarb remain in the root of BOS as contrasted to that of GLA. Differences in foliage levels of unmetabolized thiobencarb no longer exist, although the previous study showed that the concentration of label was significantly greater in BOS. By 12 DAT, metabolism patterns within roots and foliage are similar, though BOS continues to show significantly greater levels of unmetabolized $^{14}$C-thiobencarb. Throughout this study, a trend toward greater metabolism of the parent compound within the roots of GLA exists, with a corresponding decrease in the movement of parent $^{14}$C-thiobencarb to the foliage; the site of inhibition. This is especially evident in the first day after treatment. The ability to metabolize the parent molecule within the roots, may account for the tolerance observed within this cultivar.
At no time was a $^{14}$C-thiobencarb sulfoxide identified in either lettuce cultivar, nor was it found in earlier studies in rice or barnyard millet (14). The lack of any measurable amounts of sulfoxide indicates that the metabolism of thiobencarb differs from other members of the thiocarbamate chemical family, most notably EPTC and pebulate (6). The lack of sulfoxide production and its corresponding herbicidal activity indicates this is not a mechanism of selectivity with this herbicide.

The susceptible cultivar exhibited decreased leaf dry weight and leaf malformations soon after treatment with thiobencarb. Injury symptoms indicate that the herbicidal effects occur early in leaf development, probably at the developing leaf primordia on the meristem. The uptake of $^{14}$C-thiobencarb from nutrient solution was greater in BOS, probably due to its larger root system. Concentrations of label, as well as the percentage of parent thiobencarb found in developing leaves was greater in the susceptible cultivar. It is hypothesized that the larger root system of BOS at the time of treatment leads to greater uptake of the herbicide. Greater amounts of unmetabolized thiobencarb in the roots of BOS due to decreased metabolism, result in greater movement to the foliage and the site of inhibition. Hence, it is likely a combination of uptake, movement and metabolism which
accounts for the mechanism of selectivity to thiobencarb between these two cultivars.
LITERATURE CITED


GENERAL SUMMARY AND CONCLUSIONS

Thiobencarb [S-(4-chlorobenzyl) N,N,-diethylthiocarbamate] is a thiocarbamate herbicide used extensively in rice production and recently has been tested on muck grown lettuce in Ohio, Wisconsin and Florida. Weed control results have been erratic at best but differences in lettuce cultivar response to thiobencarb have been noted in field tests. Concern over differential tolerance observed in the field led to the testing of lettuce cultivars for thiobencarb tolerance.

Nutrient solution assays were utilized to eliminate the many variables associated with field testing. A nutrient solution was adapted from a nutrient film technique utilized for growing floriculture crops. The advantage of this solution over the many modified Hoaglands solutions currently used was its ease of preparation. Chelated micronutrients were added in an already prepared soluble fertilizer rather than adding the individual salts. Plants were grown in modified styrofoam cups with fitted cheesecloth bases that provided good support throughout the 12 days of the study. Adding nutrient solution containing herbicide to each individual cup may allow for increasing the number of treatments and better randomization than
utilizing large reservoir tanks. Due to the limited reservoir, however, nutrients must be at optimum levels and in readily available forms.

Five lettuce cultivars were tested in nutrient culture for thiobencarb tolerance at rates up to 6 uM using the bioassay technique just described. 'Slobolt', 'Valmaine', and 'Bibb' were found to have intermediate levels of tolerance. 'Dark Green Boston' (BOS) was found most susceptible, agreeing with observations made in the field. 'Great Lakes 366' (GLA), was the most tolerant, with significant differences between these two cultivars at 2, 3, and 4 uM. These two cultivars were tested in muck soil assays with similar levels of tolerance observed.

Studies were performed to determine the mechanism of tolerance to thiobencarb. Growth studies indicated that 4 day old BOS seedlings had 50% greater root biomass as compared to GLA. As soon as four days after adding thiobencarb to the nutrient solution, foliar growth inhibitions were observed in BOS as compared to untreated controls. In addition to decreases in foliar dry weight, leaf fasciations, including cupped and tubular leaves, were also observed in the susceptible cultivar. Since the primary site of inhibition was the developing foliage, it is hypothesized that the growth reduction and leaf fasciations came about at an early stage of leaf primordia development on the meristem.
Experiments with $^{14}$C-thiobencarb were performed to determine if differences in uptake, accumulation or metabolism may account for the tolerance differences observed. Apparently due to it's greater root system at the time of treatment, BOS took up significantly greater amounts of thiobencarb. By four days after treatment, the concentration of the label in the foliage of BOS was nearly twice that in GLA. It is at this time that significant growth reductions are occurring. Metabolism proceeded relatively quickly in both cultivars though at a faster rate in the tolerant cultivar. The decreased metabolism in the roots of BOS over time may well have led to the greater movement and accumulation in the leaves of this cultivar. By the end of this study, less than 10% of the label was in the form of the parent compound in both cultivars. Herbicide conjugates were the apparent final metabolic products. One particular metabolite, the thiobencarb sulfoxide, was not identified in these studies. This indicates that the metabolism of this compound in lettuce differs from other members of this herbicide family.

The two lettuce cultivars studied apparently differ in their early growth characteristics. The larger root system of BOS leads to greater uptake of the herbicide. Decreased metabolism of thiobencarb in the roots of this cultivar apparently leads to greater translocation and accumulation at the developing meristem. This higher concentration of
thiobencarb in the susceptible cultivar then leads to the observed growth inhibitions. Hence it is a combination of lettuce growth characteristics, uptake, accumulation, and metabolism which accounts for the selectivity of thiobencarb between these two lettuce cultivars.
LIST OF REFERENCES


