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HERBICIDAL ACTIVITY OF ETHALFLURALIN AND ITS UPTAKE AND TRANSLOCATION IN SELECTED VEGETABLE CROPS

presented by

MICHAEL DEAN WILLIS

has been accepted towards fulfillment
of the requirements for

PH.D. degree in HORTICULTURE

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HERBICIDAL ACTIVITY OF ETHALFLURALIN AND ITS UPTAKE AND TRANSLOCATION IN SELECTED VEGETABLE CROPS

By

Michael Dean Willis

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1983

ABSTRACT

HERBICIDAL ACTIVITY OF ETHALFLURALIN AND ITS UPTAKE AND TRANSLOCATION IN SELECTED VEGETABLE CROPS

by

Michael Dean Willis

This study examined differential selectivity of ethalfluralin [N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine], among cucurbit species and among bean (*Phaseolus vulgaris* L.) and cucumber (*Cucumis sativus* L.) cultivars. The fate of ethalfluralin was also determined in beans, cucumbers and peas (*Pisum sativum* L.).

In field sudies, preemergence (PRE) applications at 1.12 or 1.68 kg/ha provided excellent control of several broadleaved and grass weed species. Clear plastic mulch increased growth of transplanted muskmelons (*Cucumis melo* L.) compared to no mulch, and improved crop tolerance to ethalfluralin applied at 1.68 kg/ha preplant incorporated. Adequate crop tolerance was observed on direct seeded muskmelon as well as several cultivars of squash and pumpkin (*Cucurbita* sp.) after PRE applications of 1.12 to 1.68 kg/ha.

Injurious concentrations of ethalfluralin caused gross radial enlargement of bean root tips, swelling of hypocotyls and inhibition of hypocotyl unhooking. Inhibition of lateral root development, restric-

tion of hypocotyl elongation and hypocotyl swelling were observed in cucumbers. 'Spartan Arrow', a green bush bean, was the most sensitive of seven bean cultivars tested while 'Domino', a black turtle type bean, exhibited excellent tolerance to 9 kg/ha ethalfluralin. 'GP14A' and 'Tempo' cucumbers were most tolerant to preplant incorporated ethalfluralin at 1.12 kg/ha while 'Marketmore 70', 'Marketmore 76' and 'Calypso' were the most susceptible of 54 cultivars tested.

In field and greenhouse studies significantly more ^{14}C accumulated in leaves of cucumbers than in petioles, stems or fruits after root exposure to ^{14}C-ethalfluralin. Cucumber fruit from plants treated with 1.68 kg/ha PRE contained 0.017 ppm ^{14}C-residue while bean and pea seeds from plants treated at 1.68 kg/ha preplant incorporated contained 0.053 ppm and 0.142 ppm ^{14}C-residue respectively. ^{14}C labeled material was observed in both cucumber cotyledons and bean leaf tissue 2 h after root exposure to ^{14}C-ethalfluralin. Absorption of ^{14}C-ethalfluralin vapor was observed in both cucumber cotyledon and stem tissue. Both cucumber and bean absorbed ^{14}C-ethalfluralin topically applied to the stem and readily translocated the ^{14}C acropetally, while only limited basipital translocation occurred.

ACKNOWLEDGEMENTS

The author wishes to gratefully thank Dr. A.R. Putnam for the opportunity to pursue a dream and the invaluable experience gained under his direction. The assistance of Dr. Karen Baker in conducting much of the microautoradiography work is acknowledged. The assistance of Mr. W. Chase and my fellow students in conducting these studies was invaluable. I would like to thank Dr. S. Ries, Dr. W. Meggitt, Dr. D. Penner and Dr. H. Price for their time and effort in reviewing and critiqueing this dissertation.

A special thanks is extended to Dr. T. Golab for his technical assistance and to Lilly Research Laboratories for supplying ^{14}C-ethalfluralin and financial support.

The support obtained from the author's family made it all possible.

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INTRODUCTION

Weeds not only compete with crop plants for moisture, nutrients and light by may also interfere with crop harvesting and lower crop quality. Crops, such as cucumber (Cucumis sativus L.), which have a prostate growth habit may be at a disadvantage in competing with established weeds such as redroot pigweed (Amaranthus retroflexus L.) and common lambsquarters (Chenopodium album L.) which have an upright growth habit. The spreading vine growth and shallow root system of cucumbers makes mechanical control of weeds difficult without incurring some degree of injury to the cucumber crop.

From a practical standpoint, chemical weed control is often preferred over mechanical type control methods. Few herbicides are currently registered for use in cucurbit crops. The recent removal of CDEC (2-chloroallyl diethyldithiocarbamate) and chloramben-methyl ester (3-amino-2,5-dichlorobenzoate) from the market further limited the availablity of herbicides to the cucumber growers.

The introduction of selective postemergence graminacides such as diclofop (2-[4-(2,4-dichlorophenoxy)phenoxy] propanoic acid) may allow growers to effectively control grass type weeds in cucurbit crops. However, the chemical control of broadleaved weeds in cucurbits remains a difficult task for producers using the limited number of products currently available.

Early reports indicated that the dinitroaniline herbicide, ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluotomethyl)benzenamine) controlled many of the broadleaved and grass weed species prevalent in cucurbit production.

This study had three objectives, the first being to determine the response of selected cucurbit crops and annual weed species to soil applications of ethalfluralin, secondly, to determine if selected bean (*Phaseolus vulgaris* L.) and cucumber cultivars exhibit differential tolerance, and third, to characterize the uptake and translocation of ^{14}C-ethalfluralin in beans, cucumbers and peas (*Pisum sativum* L.).

CHAPTER 1

DINITROANILINE HERBICIDES - A LITERATURE REVIEW

HISTORY

In 1960, Alder et al. (1) first reported on the herbicidal properties of the 2,6-dinitroanilines. Since the initial disclosure of the herbicidal activity of this class of compounds by Eli Lilly Research Laboratories, many companies have developed and marketed herbicides based on similar chemistry. Although all dinitroaniline herbicides are derived from the same basic 2,6-dinitroaniline molecule, varied substitutions have lead to unique differences in both the chemical properties and biological activities of these herbicides.

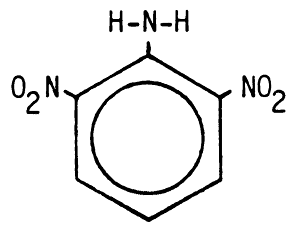
The dinitroaniline herbicides are used for selective preemergence control of annual grass and broadleaved weeds (2,61). The comparative toxicity of dinitroaniline herbicides vary greatly among plant species (8,22,31,39). Factors such as timing of applications, methods of application, depth of incorporation, soil moisture levels and rates of of dinitroaniline applications all contribute to differential selectivity of these herbicides.

PHYSICAL AND CHEMICAL PROPERTIES

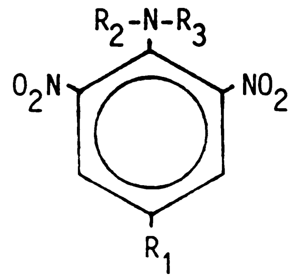
Chemical structures of selected 2,6-dinitroaniline chemistry are shown in Figure 1. With selected chemical substitutions at R1, R2 and R3, both the physical and biological properties of the compound are altered. Members of the dinitroaniline class of herbicides (Table 1) are, in general, yellow-orange, have a low water solubility, are soluble in a wide range of organic solvents and are somewhat volatile (2). The melting points of the dinitroaniline herbicides range from 42 C for fluchloralin to 152 C for nitralin and prosulfalin. Trifluralin, with a pressure of 1.99 x 10 mm Hg @ 29.5 C, is one of the more volatile dinitroaniline herbicides while nitralin exhibits one of the lowest vapor pressures at 1.8×10^{-8} mm Hg @ 25 C. Physical properties of ethalfluralin are: yellow-orange crystalline solid, faint amine odor, melting point 57-59 C, decomposes at 256 C, susceptible to decomposition by ultraviolet irradiation, vapor pressure - 8.2×10^{-5} mm Hg @ 25 C, solubility (25 C) - > 500 mg/ml in acetone, acetonitrile, chloroform, methylene chloride or xylene, 82-100 mg/ml in methanol and 0.3 ppm in water (61).

FATE IN PLANTS

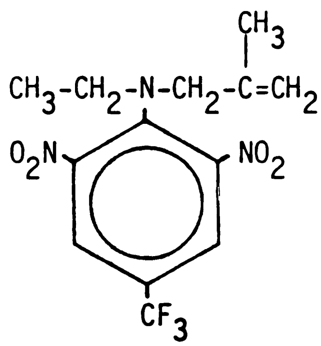
<u>Uptake and Translocation.</u> The site of maximum uptake of a dinitroaniline herbicide varies among plant species. In general, the shoot of monocots and the hypocotyl or hypocotyl hook of dicots are the major sites of dinitroaniline herbicide uptake. Barrentine and Warren (7)



Basic Structure



Points of Substitution



Ethalfluralin

Figure 1. Structure of selected 2,6-dinitroaniline chemistry.

Table 1. Common and chemical nomenclature of selected dinitroaniline herbicides.

Common Name	Chemical Name
Benefin	N-butyl-N-ehtyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine
Butralin	4-(1,1-dimethylethyl)-N-(1-methlypropyl)-2,6-dinitrobenzenamine
Dinitramine	N^4,N^4-diethyl-α,α,α-trifluoro-3,5-dinitrotoluene-2,4-diamine
Ethalfluralin	N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine
Fluchloralin	N-(2-chloroethyl)-2,6 dinitro-N-propyl-4-(trifluoromethyl)aniline
Isopropalin	2,6-dinitro-N,N-dipropylcumidine
Nitralin	4-(methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline
Oryzalin	3,5-dinitro-N^4,N^4-dipropylsulfanilamide
Pendimethalin	N-(1-ethylpropyl)-3,4 dimethyl-2,6 dinitrobenzenamine
Profluralin	N-(cyclopropylmethyl)-α,α,α-trifluoro-2,6-dinitro-N-propyl-p-toluidine
Prosulfalin	N-[[4-(dipropylamino)-3,5-dinitrophenyl]sulfonyl]-S,S-dimethylsulfilimine
Trifluralin	α,α,α-trifluoro-2,6-dinitro-N-N-dipropyl-p-toluidine

reported that the coleoptilar node of sorghum (Sorghum bicolor (L.) Moench) and the hypocotyl hook of cucumber (Cucumis sativus L.) were the most susceptible sites, as well as the sites of maximum ^{14}C-trifluralin and ^{14}C-nitralin uptake. In another study (6), they found that trifluralin was more toxic than nitralin to the shoots of sorghum and cucumber via shoot exposure while nitralin was more toxic to the roots via root exposure. Parker (43) reported that trifluralin can exert its action through shoot uptake, but root uptake appeared to be much more effective in a hybrid sorghum (S. vulgare x sudanense cv Sudax). Derr and Monaco (13) exposed shoots or roots of cucumber to ethalfluralin and found that both shoots and roots absorbed the herbicide, but exposure of roots to ethalfluralin was more toxic than shoot exposure. Jacques and Harvey (30) exposed roots or shoots of oats (Avena sativa) and peas (Pisum sativum) to vapors of ^{14}C-labeled benefin, dinitramine, fluchloralin, oryzalin, profluralin and trifluralin. They found that, except for oryzalin, all of the herbicides were absorbed by both roots and shoots of germinating oats and peas. These investigators also reported that, in general, vapor absorption was correlated with rates of herbicide volatilization. Numerous investigators have demonstrated the vapor activity of dinitroaniline herbicides (21,28,40,55). Harvey (21) suggested that vapor absorption of these herbicides may be more important than absorption from the soil solutions. However, the absorption of oryzalin vapors may be less important, because of its lower vapor pressure, than absorption from the soil solution (28,30).

The degree to which dinitroaniline herbicides are translocated in plants varies both among plant species and the herbicide compounds. For example, root applied ^{14}C-dinitramine readily translocated to the shoots of barnyardgrass (Echinochloa crus-galli (L.) Beauv.), sorghum, Palmer amaranth (Amaranthus palmeri S. Wats.) and soybean (Glycine max L. Merr.) while very little ^{14}C-profluralin was translocated (23). Jacques and Harvey (30) detected translocation of root absorbed vapors of ^{14}C-labeled benefin, fluchloralin, profluralin and trifluralin into pea shoots while little herbicide translocation was observed in oats. They reported that no shoot-root transport could be detected in either peas or oats. Little translocation of ^{14}C-trifluralin and ^{14}C-nitralin occurred after application to cucumber cotyledon or the hypocotyl hook (7). However, ^{14}C from ^{14}C-trifluralin applied as a cotyledon treatment was distributed in both cotyledons 96 h after treatment.

Strang and Rogers (54) conducted a microradioautographic study of ^{14}C-trifluralin absorption in cotton (Gossypium hirsutum L.) and soybean. They found that radioactivity from ^{14}C-trifluralin was retained primarily on the root surface in both species. Radioactivity was noted in the walls of both xylem vessels and cortical cells of roots. Little translocation out of soybean roots was observed, but limited translocation into leaves of cotton, apparently via the metaxylem was noted. Accumulation of radioactivity in the protoxylem of the cotton stem was also observed. They noted that breaks in the epidermis of the roots greatly facilitated the entrance of radioactivity into the roots.

Metabolism. As in the case of dinitroaniline herbicide translocation, the metabolism of these herbicides varies among plant species and compounds. Much of the plant metabolism work that has been reported for these herbicides was conducted by applying ^{14}C-labeled compound to the soil in which test plants were grown, after which, ^{14}C-labeled compounds extracted from the plant were identified. This experimental procedure raises a question to whether the plant metabolized the compound, if the metabolism occurred in the soil and the resulting metabolites were absorbed, or a combination of both procedures occurred. Golab et al. (18) applied ^{14}C-labeled trifluralin to greenhouse soil in which carrots (*Daucus carota* L.) were grown. The carrots were harvested 110-days after treatment, extracted with methanol and the ^{14}C-residue assayed. A majority (74.4%) of the ^{14}C was observed in the peel of the root while 25.6% was found in the root pulp. Chromatographic results showed that parent trifluralin was the major source of radioactivity in roots (89.0%) while the major conversion product was α,α,α-trifluoro-2,6-dinitro-N-(*n*-propyl)-*p*-toluidine (4.7%). In leaf extracts, only 40% of the ^{14}C appeared to be parent trifluralin while 50% of the extracted ^{14}C exhibited polar properties. Two other products, tentatively identified as α,α,α-trifluoro-5-nitro-N^4-(*n*-propyl)-toluene-3,4-diamine (1.4%) and 4-(di-*n*-propyl-amino) 3,5-dinitrobenzoic acid (4.8%), were observed in roots extracts.

Golab and Althaus (16) exposed tomato (*Lycopersicon esculentum* Mill.), pepper (*Capisicum frutescens* L.), tobacco (*Nicotiana tabacum* L.) and wheat (*Triticum aestivum* L.) to soil applied ^{14}C-isopropalin. Although the parent compound and 12 degradation products were identified in soil, no parent compound or known transformation products were identifiable in plant extracts. Only negligible amounts of ^{14}C were detected in plant tissue.

The metabolism of ^{14}C-trifluralin was examined by Biswas and Hamilton (9) in both whole plant and crude extracts of peanuts (<u>Arachis hypogaea</u> L.) and sweet potatoes (<u>Ipomoea batatas</u> (L.) Lam.). They found in both species that trifluralin was converted to compounds which were somewhat more polar in nature than parent trifluralin. Less than 1% of the total extracted ^{14}C from peanut plants was identified as trifluralin, while a greater percentage (17%) was detected in sweet potatoes. An intermediate dealkylated degradation product (α,α,α-trifluoro-2,6-dinitro-N-(<u>n</u>-propyl)-<u>p</u>-toluidine) was detected (1.2% of total ^{14}C) in whole plant extracts of peanut but not in sweet potato extracts. Two additional unidentified ^{14}C-products were found in peanuts and three unidentified compounds were found in sweet potatoes. Degradation products were also observed when crude extracts of both peanut and sweet potatoes were incubated with ^{14}C-trifluralin. These researchers suggested that a partial dealkylation of the N,N-dipropyl moiety of the trifluralin molecule preceded a reduction of the nitro-groups in the crude extracts of peanuts.

Soil-incorporated ^{14}C-benefin was absorbed into peanut and alfalfa (<u>Medicago sativa</u> L.) plants (17). After 129 days exposure, 2.33 ppm ^{14}C-benefin was incorporated into peanut plant tissue. The non-extractable radioactivity was determined in peanut stems (75%), leaves (43%), hulls (14%) and nut meats (37%). The degradation products found in the extractable ^{14}C-residues of plant tissues were essentially those products found in soil, but in very low concentrations. Significant amounts of decomposition products were found only in stem, root and hull tissue.

Plant Responses. The dinitroaniline herbicides apparently do not inhibit seed germination, even in susceptible plant species (41). However, they may inhibit growth of the entire plant in susceptible species (4). The inhibition of root development by these herbicides has been demostrated in numerous plant species by several investigators (35,38, 56). Talbert (52) and Lignowski and Scott (34) found that trifluralin inhibited mitosis in the roots of soybeans, onion (*Allium cepa* L.) and wheat. The induction of root tip swelling is often observed in herbicide treated roots (35,56). Cells in the meristematic regions of treated roots are often multinucleate (34,53,57). The exogenous applications of D-α-tocopherol acetate has been demonstrated to decrease the inhibition of tissue growth caused by dinitroaniline herbicides (2). The root swelling effect of dinitroaniline herbicides has been reduced by applications of 2,3-dimercaptopropanol (35).

Moreland and Huber (37) reported that 12 dinitroaniline herbicides were shown to interfere with electron transport and phosphorylation in isolated mung bean (*Phaseolus aureus* Roxb.) mitochondria. All of the compounds tested inhibited the rate of valinomycin-induced mitochondrial swelling in isotonic KCl within the same concentration range that caused inhibition of state three respiration. They postulated that dinitroaniline herbicides partition into the inner mitochondrial membrane, thereby decreasing membrane fluidity, and altering membrane permeability.

Barns and Krieg (5) noted a reduction in the photosynthetic rate of tomatoes after applications of trifluralin.

Schultz et al. (53) reported that synthesis of RNA, DNA and protein was suppressed in corn (Zea mays L.) root tips germinated in trifluralin but no significant effect was observed in the shoots. Penner and Early (47) found that trifluralin markedly reduced RNA synthesis based on analysis of isolated chromatin from treated corn seedlings. They postulated that the trifluralin was bound to the chromatin thus causing a reduction of template availablity for transcription. Trifluralin was not observed to inhibit chromatin activity in soybean seedlings.

FATE IN SOILS

Once a herbicide is applied to the soil, losses in activity can occur through volatility, leaching, adsorption, photodegradation, microbial degradation and chemical degradation.

Volatility. Because of high volatility, major losses in activity can occur with many dinitroaniline herbicides. Bradsley et al. (12) reported that vapor loss of trifluralin increased with herbicide concentration and soil moisture content and that losses decreased with subsurface placement in soil. Parochetti and Hein (45) compared the volatility of nitralin, trifluralin and benefin from a loamy sand under varying conditions of temperature and soil moisture. They found that volatility of both trifluralin and benefin increased with increasing temperatures of 30, 40 and 50 C and with increasing soil moisture from air dryness to field capacity. In a laboratory study, no volatilization of nitralin was detected but 24.5% and 12.5% of trifluralin and benefin, respectively, volatilized from a loamy sand after 3 h at 50 C and with soil moisture at field capacity. In a later study, Parochetti et al. (44) evaluated the volatility of 11 dinitroaniline herbicides from three different soil types. They reported no detectable volatility of nitralin or oryzalin in their 3 h laboratory study with a moist sandy soil while vapor losses of benefin, profluralin and trifluralin approached 25% under the same conditions. Soil type influenced not only the degree of volatilization but also the relative rate of volatility among dinitroaniline herbicides. Kennedy and Talbert (32) assessed the effects of soil moisture on volatilization losses of several surface applied dinitroaniline herbicides from a Taloka silt loam. Losses of butralin, nitralin, oryzalin and isopropalin after 24 h were not increased when soil moisture was increased from 1% to 14%, 26% or 33%. The loss of fluchloralin (9%), benefin (20%) and trifluralin (17%) at 1% soil moisture increased to 80%, 89% and 94%, respectively, at 33% soil moisture. The losses of dinitramine and profluralin also increased with increasing soil moisture. In compounds affected by soil moisture, the trifluoro-methyl group was a common structural component. Savage (50) reported that over an 8-day period, significantly more trifluralin and ethalfluralin volatilized from a Bosket sandy loam when the moisture content was equivalent to field capacity than when the soil was either air-dried or flooded. Losses of dinitroaniline herbicides from volatilization can be reduced by incorporating the herbicide into the soil (32,51), in fact manufacturer's label instructions require incorporation to maximize herbicidal activity.

Photodegradation. The dinitroaniline herbicides are generally considered sensitive to UV irradiation as a result of studies conducted on glass surfaces, in solvents and as a vapor (20,33,36,51). The importance of photodecomposition of soil applied dinitroaniline herbicides is not totally resolved. Wright and Warren (60) found that trifluralin photodegraded on soil surfaces but at a rate much reduced from that on a glass surface. In a later study, Parochetti and Hein (45) irradiated soil treated with trifluralin, benefin or nitralin for either 24 h or 72 h. They found no positive evidence for photodecomposition and speculated that the uneven soil surfaces provide protection from direct radiation thus greatly minimizing photodecomposition. Kennedy and Talbert (32) exposed soil coated TLC plates treated with dinitroaniline herbicides to UV light for 24 h while maintaining the ambient temperature at 5 C to minimize volatility. They found dinitramine to be greatly affected by UV light while no significant differences were found among benefin, butralin, fluchloralin, isopropalin, nitralin, oryzalin, profluralin and trifluralin.

Soil Movement. Herbicides can move in soil systems either as vapors or as solutes in the soil water phase. The degree to which a compound is adsorbed to soil, volatilized, or soluble in water determines the extent to which it moves in the soil system. Although the dinitroaniline herbicides are structurally related, they behave differently in the soil system. Several researchers have shown that trifluralin movement in soil is primarily by vapor diffusion (10,11, 27). The rate of diffusion was found to be dependent upon relative soil moisture and air-filled porosity. Jacques and Harvey (27) reported that diffusion of trifluralin, profluralin and benefin in a Plano silt loam decreased as soil water increased. The diffusion of dinitramine and fluchloralin did not change with soil water content. Oryzalin reached highest mobility in a water saturated soil, though none of the herbicides moved more than 10 mm in the soil over a 17-day period.

The dinitroaniline herbicides are not readily leached in soil, therefore, loss by this mechanism is not considered important (3,42,61). Weber (58) reports that dinitroaniline herbicides exist in the undissolved form in the soil and become associated with the lipophilic fraction of organic soil colloids. Once bound, these herbicides have greatly reduced biological activity. Millier et al. (3) reported that when trifluralin, nitralin and benefin were applied for five consecutive years as soil-incorporated treatments to cotton, herbicide residues were confined to the tilled zone of soil (upper 30 cm), and about 80 % of the residue was in the upper 15 cm of soil.

Adsorption. The binding of dinitroaniline herbicides to soils and resulting reduction in their phytotoxicities is directly related to the organic matter content of the soil (24,25,48). Hollist and Foy (25) found that in the case of trifluralin, anion exchange capacity was a better parameter than cation exchange capacity for predicting the potential of an adsorptent to reduce phytotoxicity.

Microbial/Chemical Degradation. The dinitroaniline herbicides are relatively nonpersistant in soil, with most biological activity dissipating within six months under warm, hunid conditions (20,27,42,50). Gingerich and Zimdahl (14) found that both isopropalin and oryzalin dissipated more rapidly under anaerobic than aerobic soil conditions. Trifluralin also degraded more rapidly under anaerobic conditions (46, 49). Willis et al. (59) associated this effect to the redox potential in anaerobic soil systems. The degradation of dinitroaniline herbicides in soil systems is directly correlated with temperature and soil moisture content (29,62).

The chemical degradation pathways of dinitroaniline herbicides in soil systems appears to be complex. Golab et al. (15,19) identified 28 degradtion products of trifluralin and postulated a complex set of pathways for trifluralin transformation in field soil. They also indicated that the trifluralin degradation product, α,α,α-trifluoro-toluene-3,4,5-triamine, appeared to be a key compound in the formation of soil-bound residues. Numerous soil degradation products have also been identified for other dinitroaniline herbicides (16,17).

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CHAPTER 2

RESPONSE OF SELECTED CUCURBIT CROPS AND ANNUAL WEED SPECIES TO SOIL APPLICATIONS OF ETHALFLURALIN

ABSTRACT

Three years of field research were conducted at several locations to evaluate efficacy of ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) as a selective herbicide applied either preplant incorporated (PPI) or as a preemergence (PRE) soil surface application in cucurbit crops. Clear plastic mulch increased growth of transplanted muskmelons (Cucumis melo L.) compared to no mulch, and improved crop tolerance to ethalfluralin applied at 1.68 kg/ha PPI. Injury was not observed on direct seeded muskmelons treated with PRE applications of ethalfluralin at 1.12 or 1.68 kg/ha. Direct seeded cucumbers (Cucumis sativus L.) were not injured by PRE applications of ethalfluralin at 1.12 to 2.52 kg/ha. Although direct seeded watermelon [Citrullus lanatus (Thumb.) Matsum. & Nakai] stands were reduced by 1.68 kg/ha (PRE), yields were not reduced from those of the untreated control. Direct seeded squash and pumpkin (Cucurbita sp.) were not injured by PRE applications of ethalfluralin at 1.12 or 1.68 kg/ha. Ethalfluralin applied PPI at 1.68 kg/ha afforded excellent control of common purslane (Portulaca oleracea L.). PRE applications at 1.12 or 1.68 kg/ha also resulted in excellent control of common purslane, common lambsquarters (Chenopodium album L.), redroot pigweed

(*Amaranthus retroflexus* L.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.].

INTRODUCTION

Cucumber (Cucumis sativus L.) production in Michigan is primarily for the processing market. The prolonged cool spring weather typical of Michigan, places fresh market production of muskmelon (Cucumis melo L.) and other cucurbit crops at a disadvantage with the more southern production areas. The use of clear plastic mulch has been shown by Jacobson (11) to raise soil temperatures and by other investigators (5,6) to often provide larger crop responses than black plastic mulch. A major disadvantage of using clear plastic is the need to use a herbicide under the mulch for control of weeds.

Weeds in open fields can often rapidly overgrow the cucurbit crop and successfully compete for light, nutrients and moisture. Menges and Tamez (13) reported that weed competition can reduce cucumber yields by 36% when weeds are allowed to interfere for four weeks after crop emergence. Mechanical weed control is of limited practical use because the shallow spreading root system of cucurbit crops are subject to cultivation injury and the trailing vine growth limits cultivation to an early season practice.

Only a few herbicides are currently available for weed control in cucurbit crops, due partially to the sensitive nature of cucurbits to herbicides. Differential cultivar tolerance to herbicides has also been reported in various cucurbit crops by several investigators (1,7, 14,15,17). The herbicides currently used in these crops include naptalam (N-1-naphthylphthalamic acid), bensulide [O,O-diisopropyl-phosphoro dithioate S-ester with N-(2-mercaptoethyl)benzenesulfonamide], DCPA (dimethyl tetrachloroterephthalate), dinoseb (2-sec-butyl-4,6-dinitro-phenol), and trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine). These herbicides often do not provide adequate weed control and, at times, may cause injury to cucurbit crops (2,3,8, 9,13,15). Raboy and Hopen (16) investigated the use of starch xanthide formulations of chloramben (3-amino-2,5-dichlorobenzoic acid) as a possible means of improving both effectiveness and crop tolerance. Boldt (4) reported both excellent cucumber tolerance and good control of certain grass species was obtained when dicloflop (2[4-(2,4-dichlorophenoxy)phenoxy]propanic acid) was applied postemergence.

The dinitroaniline herbicide, ethalfluralin [N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine] has been studied by several investigators (2,8,9,10,12,14,18) for its usefulness in cucurbits. Preplant incorporated treatments were observed to reduce cucumber tolerance compared to preemergence surface applications (10, 12,18).

These studies were initiated to determine (a) if preplant incorporated or preemergence surface applications of ethalfluralin influenced plant growth or yield of transplanted muskmelon, (b) if application of ethalfluralin under plastic mulch would influence tolerance of transplanted muskmelon to ethalfluralin and (c) if preemergence surface applications of ethalfluralin could provide acceptable weed control in a wide variety of cucurbit crops without producing excessive crop injury.

METHODS AND MATERIALS

Experiments were conducted at two locations: I- Clarksville Horticulture Farm (Dryden sandy loam, O.M.- 2.2%), II-Horticultural Research Center, (Metamora sandy loam, O.M.- 1.5%). Annual weed species evaluated were large crabgrass [*Digitaria sanguinalis* (L.) Scop.), common lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.), redroot pigweed (*Amaranthus retroflexus* L.), common ragweed (*Ambrosia artemisiifolia* L.) and sheperdspurse [*Capsella bursa-pastoris* (L.) Medik.]. Vegetable crops included were cucumbers (*Cucumis sativus* L. cvs Green Star and Marketmore 70), muskmelon (*Cucumis melo* L. cv Gold Star), pumpkin (*Cucurbita pepo* L. cv Howden), zucchini type summer squash (*Cucurbita pepo* L. cv Elite), acorn winter squash (*Cucurbita pepo* L. cv Table Queen), butternut winter squash (*Cucurbita moschata* L. cv Waltham Butternut) and watermelon [*Citrullus lanatus* (Thumb.) Matsum. & Nakai cv Summer Festival].

All treatments were applied with a CO_2 powered hand held plot sprayer that delivered 337 l/ha at 2.8 kg/cm^2. Plots at all locations were irrigated when plants began to show moisture stress. All experiments were designed as randomized complete blocks with either three or four replications.

Muskmelon Studies

Experiment I. Herbicides (Table 1) were applied as surface applications immediately prior to transplanting muskmelon on June 4, 1979, at location I. Treatments were applied to four replications of

1.8 by 6.1 m single row plots. Weed control ratings were taken from four replicates 22 and 31 days after treatment (DAT). Fruit yields were taken 85 DAT from three replications that were maintained weed-free from 31 DAT.

Experiment II. Ethalfluralin was applied at 1.68 kg/ha and incorporated with a garden rototiller prior to applying plastic mulch treatments (Table 2) at location I on July 24, 1980. Treatments consisted of four replicates of 1.5 m by 7.6 m single row plots with 10 plants per plot. Muskmelon transplants were planted using a liquid fertilizer starter solution immediately after the treatments were applied. Crop vigor ratings were taken at 14 and 26 DAT. At 26 DAT, the number of nodes per plant, number of lateral stems per plant, stem length and mean internode length were determined for four plants per treatment in each replication. Weed control ratings were taken and mean number of fruit per plant, mean fruit weight per plant, mean fruit and mean plant weight were determined 49 DAT, with eight plants sampled per treatment in each replication.

Experiment III. Ethalfluralin was applied at 1.12 and 1.68 kg/ha as preemergence surface applications to direct seeded muskmelon at location II immediately after planting on July 9, 1981 (Table 3). Treatments were applied to 1.8 m by 6.1 m single row plots that were replicated four times. Plant stand counts were taken 17 DAT and weed control ratings made 34 DAT. All plots were maintained weed-free from 37 DAT to harvest. At 98 DAT, mean fruit per plot, mean weight per plot and mean fruit weight were determined.

Cucumber Studies

Experiment I. Herbicides (Table 4) were applied on June 16, 1979, as preemergence surface applications to direct seeded 'Green Star' cucumbers planted on June 13 in 1.8 by 6.1 m four row plots. All treatments were replicated four times. Crop injury ratings were taken at 31 and 45 DAT while weed control ratings were taken at 45 DAT. Three replicates were maintained weed-free from 45 DAT to 52 DAT after which fruits were harvested from the center two rows of each plot. The fruits were graded according to processing standards of Pickle Packers International.

Experiment II. On June 2, 1981, ethalfluralin was applied at 1.12 and 1.68 kg/ha as a preemergence surface application to direct seeded 'Green Star' cucumbers which were planted earlier the same day. The single row plots were 0.9 by 6.1 m, and replicated four times. Plant stand counts were made 17 DAT, and weed control ratings were made 34 DAT. All plots were maintained weed-free from 37 DAT to 51 DAT after which yields were taken from the entire row.

Experiment III. 'Marketmore 70' cucumbers were treated and ratings taken as described for experiment II, except that mean fruit per plot, weight of marketable size (greater than 15 cm in length) fruit and weight of undersize fruits were obtained 64 DAT.

Watermelon Study

Watermelon were planted, treated and ratings taken the same as for cucumber experiment II except that plot size was 1.8 by 6.1 m and mean fruit per plot, mean yield per plot and mean fruit weight were taken 98 DAT.

Summer Squash Study

Zucchini type summer squash plots were established and ratings taken as reported for cucumber experiment II except that mean number of fruit per plot, mean yield per plot and mean fruit weight data were taken 41, 45, 48, and 51 DAT. After emergence, all plots were thinned to achieve a 30 cm plant spacing.

Winter Squash Studies

Separate acorn and butternut squash studies were established as described for cucumber experiment II except plot size was 1.8 by 6.1 m and mean fruit per plot, mean yield per plot and mean fruit weight data were taken 120 DAT. All plots were thinned 45 DAT to achieve a 30 cm plant spacing.

Pumpkin Study

Direct seeded pumpkins were treated as described in cucumber experiment II except that 3.7 by 6.1 m plots were employed and mean fruit per plot, mean yield per plot and mean fruit weight data were collected 120 DAT. All plots were thinned to 8 plants per plot 45 DAT.

RESULTS AND DISCUSSION

Muskmelon Studies

Experiment I. Ethalfluralin at 1.25, 1.96 and 2.52 kg/ha provided commercially acceptable control of common purslane and large crabgrass 22 DAT, but control of common purslane was noticeably reduced 31 DAT (Table 1). The 1.96 and 2.52 kg/ha rates of ethalfluralin also provided acceptable control of both redroot pigweed and common lambs-

Table 1. Weed control and crop injury ratings after pretransplant surface herbicide application in muskmelons at location I in 1979.

Treatment[a]		Ratings[b] (22 DAT)					Ratings (31 DAT)			Mean Fruit Yield/Plot[c] (85 DAT)			
Herbicide	Rate	COPU	RRPW	LACG	COLQ	CIR	COPU	RRPW	COLQ	GREEN	RIPE	ROTTEN	TOTAL
	(kg/ha)									(kg)			
Weeded control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.0	17.5	2.8	36.3
Naptalam + bensulide	4.48 4.48	9.0	10.0	9.0	10.1	2.0	7.3	8.0	7.0	12.6	14.2	3.2	30.0
Naptalam + bensulide	6.72 6.72	10.0	10.0	9.5	9.8	1.3	8.0	7.3	6.8	9.7	20.2	2.1	32.0
Naptalam + dinoseb	4.48 2.24	8.0	9.5	9.0	9.8	1.3	4.8	5.5	5.3	18.6	18.8	0.9	38.3
Ethalfluralin	1.25	9.3	7.5	7.0	9.5	1.8	4.5	3.8	4.0	14.8	13.7	0.7	29.2
Ethalfluralin	1.96	9.5	8.5	8.5	9.0	3.0	6.5	4.8	5.3	15.0	13.3	0.3	28.6
Ethalfluralin	2.52	9.5	8.8	9.3	8.5	3.0	6.0	6.0	5.5	16.4	12.5	1.7	30.5
Ethalfluralin + naptalam	1.96 4.48	10.0	10.0	9.5	9.5	2.0	8.0	7.5	7.3	17.0	14.9	1.6	33.5
Diclofop	1.12	1.5	0.8	9.8	2.0	0.8	1.5	1.0	1.0	10.1	12.4	0.8	23.2

(continued)

Table 1. Continued

Treatment[a]		Ratings[b] (22 DAT)					Ratings (31 DAT)			Mean Fruit Yield/Plot[c] (85 DAT)			
Herbicide	Rate	COPU	RRPW	LACG	COLQ	CIR	COPU	RRPW	COLQ	GREEN	RIPE	ROTTEN	TOTAL
	(kg/ha)									(kg)			
Diclofop	2.24	1.3	1.0	10.0	1.3	0.8	0.5	0.3	0.8	10.1	10.3	0.7	20.1
Diclofop + naptalam	1.12 4.48	7.5	9.0	10.0	5.8	1.0	5.8	5.0	5.3	15.7	15.2	1.4	32.3
Ethalfluralin + dinoseb	1.12 2.24	9.0	9.3	9.5	8.3	1.5	6.8	5.5	5.8	17.2	19.9	0.0	37.1
Diclofop + dinoseb	1.12 2.24	7.8	7.0	9.8	7.0	1.3	3.5	2.5	2.5	18.3	17.3	1.9	37.5
Dinoseb	2.24	8.3	5.8	8.3	7.5	1.5	2.3	1.3	1.3	17.1	15.9	2.0	35.0
LSD (0.05)		1.9	1.7	1.2	2.0	2.0	1.4	1.7	1.6	NS	8.8	2.5	17.4

[a]Herbicides applied and crop transplanted on June 4, 1979.

[b]0= no crop injury or weed control, 10= complete weed control or crop eradication. DAT= days after treatment, COPU= common purslane, RRPW= redroot pigweed, COLQ= common lambsquarters, LACG= large crabgrass, CIR= crop injury rating.

[c]Data for fruit weights are mean weights from three replicates maintained weed-free after July 5, 1979.

quarters 22 DAT, but were less effective 31 DAT. The ethalfluralin + naptalam and naptalam + bensulide treatments provided the best overall control of the weed species present in this test. The 1.96 and 2.52 kg/ha ethalfluralin treatments caused visible crop injury compared to the weeded control, but were no worse than the naptalam + bensulide or dinoseb treatments. The total and immature fruit yield from all treatments were similar to the yield of the weeded control. The ripe fruit yields from all ethalfluralin and ethalfluralin combination treatments were similar to the weeded control. Fewer rotten fruits were harvested from the 1.96 kg/ha ethalfluralin (0.3 kg) and ethalfluralin + dinoseb (0.0 kg) treatments than from the weeded control (2.8 kg).

These data indicate that effective control of common purslane, redroot pigweed, common lambsquarters and large crabgrass can be obtained with 1.96 to 2.52 kg/ha surface applied ethafluralin on a sandy loam soil. At these rates, limited crop injury occurred, but total fruit yield was not significantly reduced from that of hand weeded controls.

Experiment II. Muskmelon plants grown with clear plastic mulch in the absence of ethalfluralin produced on the average, 4.33 fruits per plant which was significantly greater than any other treatment (Table 2). While the application of ethalfluralin under clear mulch did not reduce crop vigor or reduce mean number of nodes per plant, mean stem length or mean internode length, a reduction in mean number of lateral stems per plant was observed compared to clear mulch treatment. Phytotoxic response of muskmelon to ethalfluralin was observed as a reduction in plant vigor.

Table 2. Response of transplanted muskmelon and weeds to ethalfluralin- mulch combinations at location I in 1980.[a]

Treatment	Vigor[b] Rating 14 DAT	Vigor[b] Rating 24 DAT	Growth (26 DAT) Mean nodes/ plant	Growth (26 DAT) Mean lat/ plant	Growth (26 DAT) Mean stem length	Growth (26 DAT) Mean inter length	Yield (49 DAT) Mean plant fresh wt	Yield (49 DAT) Mean fruit/ plant	Yield (49 DAT) Mean yield plant	Ratings[b] (49 DAT) COPU	Ratings[b] (49 DAT) COLQ
			(no.)	(no.)	(cm)	(cm)	(kg)	(no.)	(kg)		
Unmulched	5.5	7.5	14.9	1.3	67.9	4.6	1.00	1.18	.35	3.5	3.5
Clear mulch	0.0	0.0	21.7	9.0	138.5	6.4	4.13	4.33	2.85	0.0	0.0
Black mulch	3.0	3.5	18.2	5.2	100.5	5.5	3.10	3.00	1.39	9.0	9.2
Unmulched + ethalfluralin	6.3	8.0	13.6	1.0	62.6	4.7	.71	.85	.21	9.0	7.8
Clear mulch + ethalfluralin	.8	1.3	20.4	6.9	120.3	5.9	3.31	3.58	2.25	9.2	9.2
Black mulch + ethalfluralin	6.0	6.0	16.7	3.9	87.3	5.1	2.51	2.40	.82	9.8	9.8
LSD (0.05)	2.3	1.9	2.6	2.0	22.2	.8	.72	.67	.75	.8	.7

[a]Ethalfluralin applied at 1.68 kg/ha pretransplant incorporated and crop transplanted on July 24, 1980. Data for nodes/plant, lateral/plant, stem length and internode length are means for four replicates with four plants sampled per replicate. Data for vigor rating, plant fresh wt, fruit plant and yield/plant are means for four replicates with eight plants sampled per replicate.

[b]0= no reduction in crop vigor or weed control, 10= complete weed control or complete inhibition of crop growth. DAT= days after treatment, lat= lateral stems, inter= internode, COPU= common purslane and COLQ= common lambsquarters.

Commercially acceptable control of common purslane and common lambsquarters was obtained with the application of 1.68 kg/ha ethalfluralin under clear mulch while severe weed growth was observed in the absence of herbicide. The weed growth under the clear mulch caused it to lift, and resulted in punctures and partial loss of anchoring of the plastic. Soil temperatures under the clear mulch were observed at times to be 5.5°C higher than temperatures under black mulch or the bare soil treatments. Plant growth and fruit yields in the ethalfluralin treatments were generally somewhat lower than in the comparative non-ethalfluralin treatments.

These data support previous studies (5,6,11), which indicate that the culture of muskmelons on plastic mulch can provide significant yield advantages over bare ground culture. This observed growth advantage, under plastic mulch culture, was most likely due to increased soil temperature, increased retention of soil moisture and a reduction in soil compaction due to rainfall. Although the highest production was obtained in the clear mulch treatment, weed infestation caused unacceptable lifting and tearing of the plastic mulch. Based on this study, it is not possible to determine to what degree the competition effects of weeds in the clear plastic mulch treatment influenced muskmelon growth. However, it would be reasonable to expect conditions such as higher weed pressure, earlier weed competition or drought to to amplify the deleterious effects of weed competition under clear plastic mulch on muskmelon growth and yield. The use of ethalfluralin under clear plastic prevented serious infestations of common purslane and common lambsquarters. Significantly higher fruit yields were observed in the ethalfluralin + clear plastic treatment than either the unmulched or black plastic mulch treatment.

Experiment III. Preemergence surface applications of ethalfluralin at 1.12 and 1.68 kg/ha on direct seeded muskmelon gave commercially acceptable control of redroot pigweed, common lambsquarters and common purslane while not causing a reduction in crop stand as compared to untreated controls (Table 3). The mean fruit yield per plot (48.0 and 50.3 kg/ha respectively) observed for the 1.12 and 1.68 kg/ha ethalfluralin treatments were about 60% greater than that obtained in untreated controls. The reduction in yields obtained in the untreated controls, compared to the ethalfluralin treatments, are most likely attributable to the early competitive effects of the high weed populations observed in this muskmelon study.

Cucumber Studies

Experiment I. There was no significant crop injury in any of the ethalfluralin treatments at either the 31 or 45 DAT rating period (Table 4). Although weed densities were low throughout all the plots in this experiment, a significant increase in weed control was observed with the higher rates of ethalfluralin as compared to the 1.25 kg/ha. There were no differences in any of the yield parameters measured between the untreated control and the singular ethalfluralin treatments. The increasing rates of ethalfluralin appeared to provide earlier yields possibly due to reductions in early season weed competition. The ethalfluralin + naptalam treatment resulted in lower total fruit yield (10.2 kg) when compared the untreated control (17.8 kg).

The preemergence surface applications of ethalfluralin at all rates did not reduce total fruit yields from those obtained in untreated plots.

Table 3. Response of seeded muskmelon to preemergence surface applications of ethalfluralin at location II in 1981.

Treatment[a]			Ratings[b] (34 DAT)			Yield (98 DAT)	
Herbicide	Rate	Plants /plot (17 DAT)	RRPW	COLQ	COPU	Mean fruit /plot	Mean yield /plot
	(kg/ha)	(no.)				(no.)	(kg)
Untreated		25.8	0.0	0.0	0.0	29.0	30.0
Ethalfluralin	1.12	26.8	8.1	9.4	9.5	42.0	48.0
Ethalfluralin	1.68	27.5	8.6	9.5	9.5	43.5	50.3
LSD (0.15)		NS	.9	.3	.1	13.7	14.0

[a]Crop planted and ethalfluralin applied on June 2, 1980. All plots were maintained weed-free after July 9, 1981.

[b]0= no weed control, 10= complete weed control. DAT= days after treatment, RRPW= red root pigweed, COLQ= common lambsquarter, COPU= common purslane.

Table 4. Weed and pickling cucumber response to preemergence herbicide applications at location I in 1979.

Treatment		Ratings[b] (31 DAT)	Ratings[b] (45 DAT)						Cucumber yield/plot[c] (52 DAT)				
Herbicide[a]	Rate	CIR	CIR	COLQ	RRPW	CORW	COCW	SHPU	#1	#2	#3	Over-sized	Total
	(kg/ha)								(kg)				
Untreated		0.0	0.0	0.0	0.0	0.0	0.0	0.0	.15	1.0	4.1	12.1	17.8
Naptalam + bensulide	4.48 4.48	1.5	1.3	6.8	7.8	8.0	6.8	7.0	.41	1.2	4.4	9.3	15.4
Naptalam + bensulide	6.72 6.72	1.8	1.8	7.5	8.8	7.8	8.0	7.3	.35	1.3	4.2	11.6	17.3
Naptalam + dinoseb	4.48 2.24	0.5	1.5	8.5	8.5	9.0	8.3	9.0	.51	0.7	5.3	11.0	17.5
Ethalfluralin	1.25	0.0	0.0	0.3	0.3	0.5	1.0	1.3	.27	1.0	3.8	12.6	17.8
Ethalfluralin	1.96	0.0	0.3	5.0	5.0	6.3	5.8	4.0	.24	1.1	4.7	13.6	19.6
Ethalfluralin	2.52	0.0	0.0	6.8	6.8	6.8	5.3	7.0	.18	0.9	6.0	15.5	22.5
Ethalfluralin + naptalam	1.96 4.48	1.3	1.3	7.8	7.5	4.8	7.5	7.5	.44	1.2	3.4	5.3	10.2
Diclofop	1.12	0.0	0.3	1.3	1.5	2.5	2.3	2.8	.23	0.8	3.5	9.8	14.3

(continued)

Table 4. Continued

Treatment		Ratings[b] (31 DAT)	Ratings[b] (45 DAT)						Cucumber yield/plot[c] (52 DAT)				
Herbicide[a]	Rate	CIR	CIR	COLQ	RRPW	CORW	COCW	SHPU	#1	#2	#3	Over-sized	Total
	(kg/ha)								(kg)				
Diclofop	2.24	0.5	0.3	1.0	1.0	1.0	1.8	1.5	.20	1.2	4.9	9.0	15.3
Diclofop + naptalam	1.12 4.48	1.5	0.5	5.5	6.3	6.0	6.3	6.3	.33	1.2	3.9	10.2	15.6
Ethalfluralin + dinoseb	1.12 2.24	0.8	0.5	7.5	4.0	7.5	6.3	6.5	.35	1.0	2.7	11.4	15.4
Diclofop + dinoseb	1.12 2.24	0.3	0.5	6.8	5.8	8.3	7.3	7.3	.27	1.2	4.3	11.2	17.0
Dinoseb	2.24	1.0	1.0	7.8	6.0	8.0	7.5	7.0	.23	0.8	2.2	14.5	19.7
LSD (0.05)		1.7	1.3	1.3	2.2	2.0	1.8	1.8	.18	NS	1.9	6.0	7.0

[a]Crop planted on June 13 and herbicides applied on June 16, 1979.

[b]0= no crop injury or weed control, 10= complete weed control or crop eradication. DAT= days after treatment, CIR= crop injury ratings, COLQ= common lambsquarters, RRPW= redroot pigweed, CORW= common ragweed, COCW= common chickweed, SHPU= shepherdspurse.

[c]Data for cucumber yields are means from three replicates maintained weed-free after July 31, 1979.

Weed populations were probably too low in this study to allow valid conclusions regarding proper herbicide rates.

Experiment II. Preemergence surface applications of ethalfluralin at 1.12 and 1.68 kg/ha provided commercially acceptable control of redroot pigweed, common lambsquarters and common purslane under conditions of high weed populations while cucumber stands were not adversely effected (Table 5). Yield of #1 cucumbers increased to 313 kg for the 1.68 kg/ha ethalfluralin treatment compared to 175 kg obtained in the untreated control. Although no differences were observed among treatments in yields of Grade #2, Grade #3, or oversized fruit, both ethalfluralin treatments had total yields that were significantly greater than that of the untreated controls. It appears that early weed competition in the untreated control caused a delay in earliness of fruit development compared to the ethalfluralin treatments.

Preemergence surface application of ethalfluralin at 1.12 and 1.68 kg/ha on the cultivar 'Green Star' provided control of the major weed species present with no reduction in crop stand. Plots treated with ethalfluralin significantly out-yielded untreated controls by about 80%.

Experiment III. Ethalfluralin applied as PRE surface treatments of 1.12 and 1.68 kg/ha to the slicing ('Marketmore 70') cucumber provided commercial control of the major weed species present with no reduction in cucumber stand (Table 6). Both ethalfluralin treatments produced significant increases in marketable and total fruit yields compared to the untreated control. As was noted in cucumber experiment II, the early weed competition in the untreated cucumber plots

Table 5. Response of weeds and seeded pickling cucumbers to preemergence surface applications of ethalfluralin at location II in 1981.

Treatment[a]			Ratings[b] (34 DAT)			Mean yield/plot (51 DAT)				
Herbicide	Rate	PLants /plot 17 DAT	RRPW	COLQ	COPU	Grade #1	Grade #2	Grade #3	Over-sized	Total
	(kg/ha)	(no.)				(g)				
Untreated		19.8	0.0	0.0	0.0	175	1207	1956	957	4295
Ethalfluralin	1.12	22.8	8.6	8.8	9.5	262	1370	3705	2777	8114
Ethalfluralin	1.68	20.3	9.1	9.0	9.5	313	1538	3758	2324	7933
LSD (0.05)		NS	.8	.4	.1	103	NS	NS	NS	3145

[a]Crop planted and ethalfluralin applied on June 2, 1980. All plots were maintained weed-free after July 9, 1981.

[b]0= no weed control, 10= complete weed control. DAT= days after treatment, RRPW= redroot pigweed, COLQ= common lambsquarters, COPU= common purslane.

Table 6. Response of weeds and seeded slicing cucumbers to preemergence surface application of ethalfluralin at location II in 1981.

Treatment[a]			Ratings[b] (34 DAT)			Mean yield/plot (64 DAT)		
Herbicide	Rate	Plants /plot (17 DAT)	RRPW	COLQ	COPU	Market-able	Under-size	Total
	(kg/ha)	(no.)				(kg)		
Untreated		23.3	0.0	0.0	0.0	13.7	1.1	14.8
Ethalfluralin	1.12	20.0	8.9	9.3	9.5	20.3	1.0	21.3
Ethalfluralin	1.68	20.8	8.9	9.4	9.4	22.7	.9	23.6
LSD (0.05)		NS	.6	.3	.3	5.7	NS	5.8

[a] Crop planted and ethalfluralin applied on June 2, 1980. All plots were maintained weed-free after July 9, 1981.

[b] 0= no weed control, 10= complete weed control. DAT= days after treatment, RRPW= redroot pigweed, COLQ= common lambsquarters, COPU= common purslane.

apparently delayed earliness of fruit development compared to the ethalfluralin treatments.

Watermelon Study

A reduction in crop stand was observed in the 1.68 kg/ha ethalfluralin treatment, but total fruit yield was not reduced from that obtained in the untreated control (Table 7), in fact, fruit yield from the 1.12 kg/ha treatment was higher than that from the controls. Acceptable weed control was provided by both ethalfluralin treatments.

Summer Squash Study

Control of redroot pigweed, common lambsquarters and common purslane was commercially acceptable with both rates of ethalfluralin while crop stand was not adversely effected (Table 8). There were no differences observed in any of the yield parameters taken at individual harvest dates. However, the season total mean yield for the 1.68 kg/ha ethalfluralin treatment was 19% higher than the untreated control.

Winter Squash Studies

Similar results were obtained in separate studies with acorn and butternut type winter squash (Table 9). While commercially acceptable control of the major weed species present was obtained, there was no differences among treatments with regard to either crop stand or fruit yield.

Pumpkin Study

As was noted for the other studies conducted at location II in 1981, the degree of weed control obtained with the ethalfluralin treatments was commercially acceptable (Table 10). There were no observed differences among the treatments in either pumpkin stand counts or in mean

Table 7. Response of weeds and seeded watermelon to preemergence surface applications of ethalfluralin at location II in 1981.

Treatment[a]			Ratings[b] (34 DAT)			Yield (98 DAT)		
Herbicide	Rate	Plants /plot (17 DAT)	RRPW	COLQ	COPU	Mean fruit /plot	Mean fruit wt	Mean yield /plot
	(kg/ha)	(no.)				(kg)		
Untreated		22.8	0.0	0.0	0.0	26.0	2.2	57.2
Ethalfluralin	1.12	21.5	8.6	9.1	9.4	28.5	3.2	91.8
Ethalfluralin	1.68	20.0	8.6	9.4	9.5	29.8	3.0	81.0
LSD (0.05)		2.7	1.3	.3	.3	NS	.8	30.9

[a]Crop planted and ethalfluralin applied on June 2, 1981. All plots were maintained weed-free after July 9, 1981.

[b]0= no weed control, 10= complete weed control. DAT= days after treatment, RRPW= redroot pigweed, COLQ= common lambsquarters, COPU= common purslane.

Table 8. Weed control and crop response after preemergence surface applications of ethalfluralin in direct seeded summer squash at location II in 1981.

Treatment[a]			Ratings[b] (34 DAT)			Yield[c]		
Herbicide	Rate	Plants /plot (17 DAT)	RRPW	COLQ	COPU	Mean fruit /plot	Mean yield /plot	Mean fruit wt
	(kg/ha)	(no.)					(kg)	(g)
Untreated		28.5	0.0	0.0	0.0	75.8	16.7	222
Ethalfluralin	1.12	29.5	9.0	9.1	9.5	85.8	18.5	216
Ethalfluralin	1.68	28.8	9.5	9.5	9.5	86.5	19.8	230
LSD (0.05)		NS	.4	.5	.0	NS	2.7	NS

[a]Crop planted and ethalfluralin applied on June 2, 1981. All plots were maintained weed-free after July 9, 1981.

[b]0= no weed control, 10= complete weed control. DAT= days after treatment, RRPW= redroot pigweed, COLQ= common lambsquarters, COPU= common purslane.

[c]Means of yields taken 41,45,48 and 51 DAT.

Table 9. Weed and crop response to preemergence applications of ethalfluralin in seeded winter squash at location II in 1981.

	Treatment[a]			Ratings[b] (34 DAT)			Yields (120 DAT)	
Type	Herbicide	Rate	Plants /plot (17 DAT)	RRPW	COLQ	COPU	Mean fruit /plot	Mean fruit /plot
		(kg/ha)	(no.)				(no.)	(kg)
Acorn	untreated		22.8	0.0	0.0	0.0	36.0	31.0
	ethalfluralin	1.12	21.5	9.1	9.1	9.3	40.8	33.8
	ethalfluralin	1.68	20.8	9.3	9.5	9.5	42.5	34.5
	LSD (0.05)		NS	.4	.3	.5	NS	NS
Butternut	untreated		26.8	0.0	0.0	0.0	34.0	49.5
	ethalfluralin	1.12	26.8	8.3	9.3	9.5	39.0	55.0
	ethalfluralin	1.68	28.8	8.6	9.1	9.5	44.0	67.5
	LSD (0.05)		NS	.9	.5	.1	NS	NS

[a]Crop planted and ethalfluralin applied on June 2, 1981. All plots were maintained weed-free after July 9, 1981. All plots were thinned to a 12 inch plant spacing on July 17, 1981.

[b]0= no weed control, 10= complete weed control. DAT= days after treatments, RRPW= redroot pigweed, COLQ= common lambsquarters, COPU= common purslane.

Table 10. Weed and crop response after preemergence applications of ethalfluralin to direct seeded pumpkins at location II in 1981.

Treatment[a]			Ratings[b] (34 DAT)			Yields (120 DAT)	
Herbicide	Rate	Plants /plot (17 DAT)	RRPW	COLQ	COPU	Mean fruit /plot	Mean fruit /plot
	(kg/ha)	(no.)				(no.)	(kg)
Untreated		16.8	0.0	0.0	0.0	13.0	115.8
Ethalfluralin	1.12	19.8	0.0	8.8	9.4	16.0	135.3
Ethalfluralin	1.68	19.5	9.0	9.1	9.5	17.8	162.0
LSD (0.05)		NS	0.7	1.0	0.6	3.9	30.2

[a] Crop planted and ethalfluralin applied on June 2, 1981. All plots were maintained weed-free after July 9, 1981. All plots were thinned to eight plants per plot on July 17, 1981.

[b] 0= no weed control, 10= complete weed control. DAT= days after treatment RRPW= redroot pigweed, COLQ= common lambsquarters, COPU= common purslane.

fruit weight. Plots receiving the 1.68 kg/ha treatment produced more fruit and more weight per plot than did the untreated controls.

Preemergence surface applications of ethalfluralin at 1.12 and 1.68 kg/ha provided efficacious control of common purslane, common lambsquarters and redroot pigweed in all tests conducted at location II in 1981. Large crabgrass was controlled with the 1.25, 1.96 and 2.52 kg/ha rates of ethalfluralin applied as a preemergence surface treatment in the muskmelon study at location I in 1979.

In summary, the use of clear plastic mulch appeared to improve the tolerance of transplanted muskmelons to preplant incorporated ethalfluralin treatments. Direct seeded muskmelons exhibited adequate tolerance to preemergence applications of ethalfluralin at 1.12 and 1.68 kg/ha.

'Green Star' cucumbers were very tolerant to PRE surface applications of ethalfluralin from 1.25 to 2.52 kg/ha on the sandy loam soil with 2.2% O.M. Both cucumber cultivars exhibited 50-90% increase in yield in the ethalfluralin treatments compared to the untreated controls in the 1981 studies. Although watermelon stand counts were reduced in the 1.68 kg/ha ethalfluralin treatments at the same site, the total yields were not reduced from those of the untreated control. Pumpkin, summer squash and winter squash were tolerant to PRE surface applications of ethalfluralin at 1.12 and 1.68 kg/ha.

Preemergence surface applications of ethalfluralin can be utilized for efficacious control of several weed species in cucurbit crops with a reasonable margin of safety. In addition, the use of clear plastic mulch can enhance growth of transplanted muskmelons and improve their tolerance to preplant incorporated ethalfluralin.

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CHAPTER 3

RESPONSE OF SELECTED CULTIVARS OF BEANS (*Phaseolus vulgaris* L.) AND CUCUMBERS (*Cucumis sativus* L.) TO SOIL APPLICATIONS OF ETHALFLURALIN

ABSTRACT

Greenhouse and incubator studies were initiated to determine if ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) causes morphological aberrations in germinating bean or cucumber seedlings and whether there is differential bean and cucumber cultivar tolerance to ethalfluralin. Ethalfluralin caused gross radial enlargement of bean root tips, swelling of hypocotyls, and in 'Spartan Arrow' inhibition of hypocotyl unhooking. Inhibition of lateral root development, restriction of hypocotyl elongation and hypocotyl swelling were observed in cucumbers treated with ethalfluralin. 'Spartan Arrow' snap beans were the most sensitive of bean cultivars tested while 'Domino', a black turtle type bean, were very tolerant to ethalfluralin at 8.96 kg/ha. Visible injury ratings were found to be more indicative of ethalfluralin injury to beans than either shoot fresh or dry weights. The 'GP14A' and 'Tempo' cucumber cultivars were found to be tolerant to preplant incorporated treatments of ethalfluralin at 1.12 kg/ha while 'Marketmore 70', 'Marketmore 76' and 'Calypso' cultivars were significantly reduced in both shoot fresh and dry weight production.

INTRODUCTION

Members of the dinitroaniline class of herbicides often cause morphological abnormalities in germinating seedlings. Lignowski and Scott (8) reported that trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) inhibited root elongation and induced root tip swelling of corn (Zea mays L.) and wheat (Triticum aestivum L.). Schultz et al. (14) also reported that trifluralin caused radial enlargement of the root tip of corn but found no effect upon germination. Upadhyaya and Noodén (16) found that root applied oryzalin (3,5-dinitro-N^4,N^4-dipropylsulfanilamide) inhibited root elongation and caused swelling in the elongation zone. In another study (15), they found that several dinitroaniline herbicides induced root tip swelling in corn seedlings. Murray et al. (10) found that dinitramine (N^4,N^4-diethyl-α,α,α-trifluoro-3,5-dinitrotoluene-2,4-diamine), profluralin (N-[cyclopropylmethyl]-α,α,α-trifluoro-2,6-dinitro-N-propyl-p-toluidine) and trifluralin reduced both root and shoot growth in cotton (Gossypium hirsutum L.) and soybean (Glycine max L.). Barrentine and Warren (2) noted that trifluralin had a greater effect on the growth of soybean (Sorghum bicolor L. Moench) and cucumber (Cucumis sativus L.) shoots while nitralin (4-(methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline) caused the greatest inhibition of root growth. They also reported that when trifluralin and nitralin were applied to cucumber hypocotyl hooks, hypocotyl swelling occurred, as well as a reduction in shoot height (3). Harvey (7) studied the relative toxicities of dinitroaniline herbicides and found that the inhibition of root and shoot growth varied among plant species tested and among herbicides. Murray et al. (11) investigated the effect of structural change on the activity of six dinitroaniline herbicides. They reported that the tolerance of several species of plants varied with structural changes in the herbicide molecule.

For decades, plant breeders have routinely screened their breeding lines and progeny for superior disease resistance. More recently, the selection of genetic material for improved herbicide tolerance has become important. The differential in cultivar tolerance to a herbicide can be significant, for example, certain cultivars of soybeans and wheat have been reported to be more tolerant to metribuzin (4-amino-6-*tert*-butyl-3-(methylthio)-as-triazin-5(4H)-one) than others (6,9,13). Freedman (5) found several potato (*Solanum tuberosum* L.) cultivars with superior tolerance to metribuzin while Werner and Putnam (17) identified a cucumber accession that tolerated 4 fold the rate of atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) that killed most other cucumber cultivars and accessions. Adeniji and Coyne (1) have proposed a mechanism for inheritance of resistance to trifluralin injury in squash (*Cucurbita moschata* Poir). In a limited screening trial, Derr and Monaco (4) found no difference in ethalfluralin tolerance among the 16 cucumber cultivars tested. In contrast, Nuland and Boyes (12) reported finding differential tolerance between cucumber cultivars 'Spartan Dawn' and 'SMR 58' to trifluralin and chloramben (3-amino-2,5-dichlorobenzoic acid).

These studies were initiated to determine (a) if ethalfluralin causes similar morphological aberrations in cucumber and bean seedlings that have been reported for other dinitroaniline herbicides in other crops, and (b) whether there is differential bean and cucumber cultivar tolerance to ethalfluralin.

METHODS AND MATERIALS

Effects of ethalfluralin on germinating beans. Germination tests were conducted by placing five bean (*Phaseolus vulgaris* L. cv Seafarer) seeds in a 10 cm diameter glass petri dish lined with one piece of Whatman #1 filter paper. The filter paper was moistened with 4.0 ml of water containing ethalfluralin at 0 or 100 ppm. The petri dishes were sealed and held in an incubator at 30 C for two days. Each treatment was replicated four times and the experiment was repeated four times. Two days after treatment, the germinated seedlings were examined for morphological variability between treatments and percent germination was recorded.

Effects of ethalfluralin on germinating cucumbers. Cucumber (*Cucumis sativus* L. cv Green Star) germination tests were conducted in a manner similar to that described for beans except the concentrations of ethalfluralin were 0, 3.51, 35.1, or 351 ppm. Morphological variability and percent germination was recorded 4 days after treatment.

Differential tolerance studies: Cultural Practices. Ethalfluralin was applied to the surface of a greenhouse soil mix (1:1:1, v/v/v, sand: peat: soil), by passing flats under a conveyor type sprayer that delivered approximately 340 L/ha spray diluent. The treated soil was removed from the flat, throughly mixed in a rolling type mixer, and then replaced into the flat. The flats were seeded and placed in a greenhouse maintained at 28±4 C day and 20±4 C night. Supplemental lighting was provided by metal halide lamps with a 16 h photoperiod and an intensity of 842 $Em^{-2}S^{-1}$. All plants were watered overhead as necessary and fertilized every five days with a dilute complete fertilizer solution. The experiment was terminated 12 (beans) and 16 (cucumbers) days after treatment by taking phototoxicity ratings and cutting the shoots off at soil level for fresh weight determination. The shoots were oven dried at 50 C for 72 h and dry weights taken. Weight data for ethalfluralin treatments are presented as percent of control. A randomized complete block design with four replicates was used for each study. The bean and cucumber studies were both conducted twice during the spring of 1982.

Bean study. For each bean cultivar tested (Table 1) 10 seeds were planted in 16 x 13 x 5 cm styrofoam flats containing soil treated with 0, 1.12, 2.24, 4.48, or 8.96 kg/ha ethalfluralin. The seeds were planted in two rows approximately 2 cm deep with 3 cm between seeds in a row and 6.5 cm between rows. All raings were made 12 days after treatment.

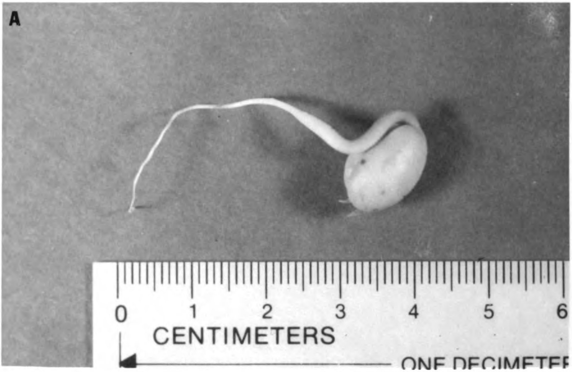
Cucumber study. The cucumber cultivars tested (Table 4) were planted into 25.5 x 25.5 x 5 cm plastic flats with soil containing either 0 or 1.12 kg/ha ethalfluralin. Six seeds for each cultivars were planted in single rows with approximately 4.5 cm between seeds in a row and 4.3 cm between each of the six rows. All ratings were made 16 days after treatment.

RESULTS AND DISCUSSION

Effects of ethalfluralin on germinating beans. After 2 days, there was no significant difference in percent germination between seeds in the 0 or 100 ppm ethalfluralin treatments. Visual examination of the seedlings treated with 100 ppm ethalfluralin (Figure 1B) revealed a consistant swelling of the root tip and of the hypocotyl region immediately below the cotyledons compared to the control (Figure 1A). As a result of gross radial enlargement of the root tip, cracks developed in the epidermis and extended into the cortex of the root. The root swelling of *Phaseolus vulgaris* seedlings caused by ethalfluralin in this study is consistant with reports by other investigators (8,10,15,14) that dinitroaniline herbicides induce root tip swelling in a wide range of plant species.

Effects of ethalfluralin on germinating cucumbers. The percent germination of cucumber seeds treated with 0. 3.51, 35.1, or 351 ppm ethalfluralin was not different four days after treatment. Previous investigators (14) have shown that dinitroaniline herbicides, as a group, do not affect the germination of seeds. The results of this study indicate that ethalfluralin does not differ from other dinitroaniline herbicides in this respect. Increasing concentrations of ethalfluralin had marked effects on lateral root development, shoot elongation, and cotyledon coloration of germinating cucumber seedlings (Figure 2). Germinating cucumber seedlings treated with 3.51 ppm ethalfluralin showed little or no inhibition of lateral root development. Lateral root development was severly restricted by 35.1 ppm and

Figure 1. Bean seedlings germinated in petri dish with ethalfluralin at 0 ppm (A) and 100 ppm (B).


A
0 1 2 3 4 5 6
CENTIMETERS

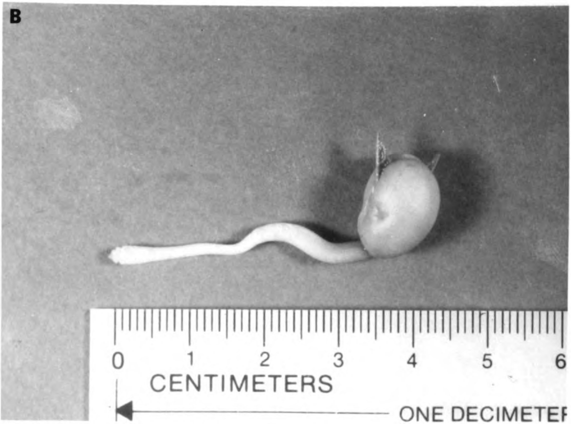
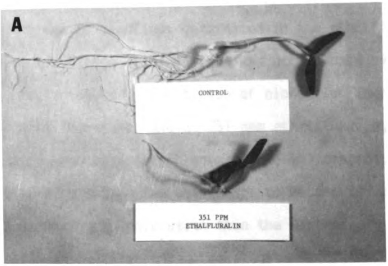
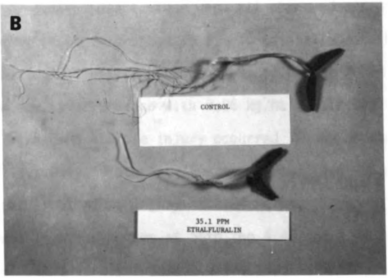
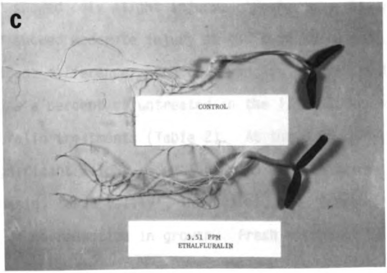

B
0 1 2 3 4 5 6
CENTIMETERS

Figure 2. Cucumber seedlings germinated in petri dishes with ethalfluralin at 0 and 351 ppm (A), 0 and 35.1 ppm (B), and with 0 and 3.51 ppm (C).


A
CONTROL
351 PPM
ETHALFLURALIN


B
CONTROL
35.1 PPM
ETHALFLURALIN


C
CONTROL
3.51 PPM
ETHALFLURALIN

usually absent with 351 ppm ethalfluralin. Hypocotyl elongation was noticeably restricted in seedlings germinated in the ethalfluralin treatments. The inhibition of elongation was accompanied by an increase in hypocotyl diameter. Inhibition of elongation increased as ethalfluralin rates increased, and at 351 ppm ethalfluralin, there was a 70% reduction in hypocotyl length compared to the control. Higher rates of ethalfluralin caused the cotyledons to become curled and to develop a much darker green coloration than the control.

Differential bean cultivar tolerance study. Reductions in plant height, mass, or general plant vigor were all observed as injury symptoms on beans grown in treated soil. Additionally, inhibition of hypocotyl unhooking and extreme hypocotyl swelling was noted in the 'Spartan Arrow' cultivar treated with 8.96 kg/ha ethalfluralin (Figure 3B). At 1.12 kg/ha, no visible injury occurred in any of the cultivars tested (Table 1). Only 'Neptune', 'Spartan Arrow', and 'Black Magic' exhibited slight symptoms of injury at 2.24 kg/ha. Though all cultivars showed injury symptoms at both the 4.48 and 8.96 kg/ha rates, 'Spartan Arrow' was severely injured, while 'Domino', 'Swan Valley', and 'Montcalm' showed only slight injury. 'Seafarer', 'Black Magic', and 'Neptune ' showed moderate injury at the 8.96 kg/ha rate. There was no significant difference among cultivars in either fresh weight or dry weight as a percent of untreated in the 1.12. 2.24, or 4.48 kg/ha ethalfluralin treatments (Table 2). At the 8.96 kg/ha rate there were significant weight reductions with some cultivars. On a fresh weight basis, 'Swan Valley', 'Montcalm', and 'Domino' cultivars showed little or no reduction in growth. Fresh weight of 'Spartan

Figure 3. 'Black Magic' (A), a tolerant bean cultivar and 'Spartan Arrow' (B), a susceptible cultivar germinated in soil with ethalfluralin incorporated at 0, 1.12, 2.24, 4.48 and 8.96 kg/ha (left to right).

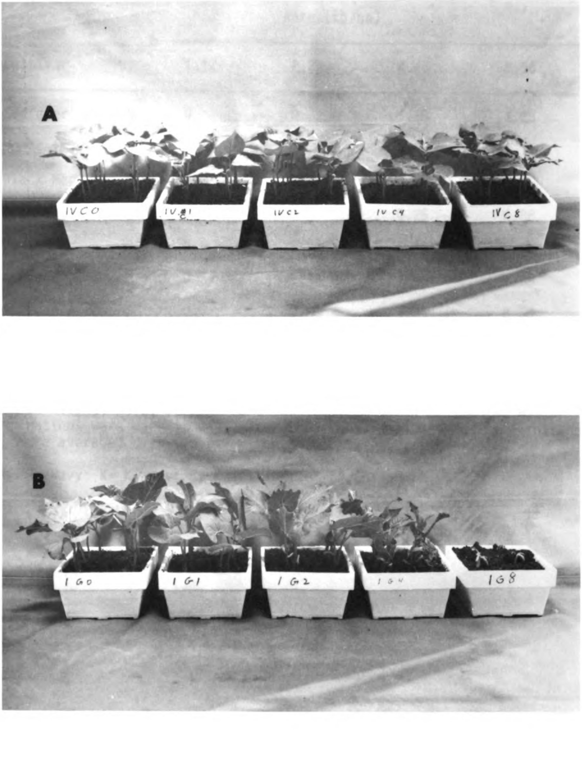

A
IV C 0
IV C 2
IV C 4
IV C 8
B
1 G 0
1 G 1
1 G 2
1 G 4
1 G 8

Table 1. Phytotoxicity ratings on bean cultivars after preplant applications of ethalfluralin.[a]

Cultivar	Bean[b] type	Rate (kg/ha) 1.12	2.24	4.48	8.96
Seafarer	N	0	0	1.38	4.25
Montcalm	K	0	0	0.50	1.12
Black Magic	B	0	0.38	0.50	2.75
Swan Valley	N	0	0	0.25	0.88
Domino	B	0	0	0.12	0.50
Neptune	N	0	1.38	1.75	2.88
Spartan Arrow	S	0	0.88	3.25	8.50
LSD (0.05)		NS	0.46	1.35	1.23

[a]Bean phytotoxicity ratings were on a 0-10 scale where 0= no effect on height, mass, or vigor and 10= complete death of plants. All ratings were made 12 days after treatment. The phytotoxicity ratings are averaged over two experiments.

[b]N= Navy; K= Kidney; B= Black Turtle; S= Snap.

Table 2. Average dry weight of untreated beans and influence of ethalfluralin on shoot dry weight production as a percent of control.[a]

		Ethalfluralin (kg/ha)			
	0	1.12	2.24	4.48	8.96
Cultivar	Shoot dry wt (g)	Shoot dry wt (% of control)			
Seafarer	.166	105.6	105.6	95.4	83.4
Montcalm	.299	100.0	90.8	85.8	87.4
Black Magic	.191	95.4	92.1	94.8	85.3
Swan Valley	.171	95.5	99.6	91.5	101.9
Domino	.178	98.1	98.8	91.0	86.6
Neptune	.176	86.9	84.0	84.6	80.3
Spartan Arrow	.232	97.6	78.0	99.5	94.5
LSD (0.05)		NS	NS	NS	13.5

[a]The average shoot dry weights per plant, taken 12 days after planting, are averaged over two experiments.

Arrow' was inhibited significantly more (44%) than any of the other cultivars tested. An intermediate reduction in fresh weight was observed with 'Neptune', 'Black Magic', and 'Seafarer'. There was no reduction in dry weight with 'Swan Valley' even with the 8.96 kg/ha treatment. Although, 'Spartan Arrow' dry weight production appeared somewhat depressed, it was not significantly lower (5% level) than that of 'Swan Valley'. The dry weight production of all other cultivars tested was 14-20% lower than that of 'Swan Valley'.

The correlation coefficients for bean phytotoxicity ratings, percent fresh weights and percent dry weights were calculated for each herbicide rate (Table 3). The correlation between the phytotoxicity rating and percent dry weight production was low at all rates tested. An example of this lack of correlation can be seen in data presented in Tables 1 and 2 and Figure 3B for 'Spartan Arrow' treated at 8.96 kg/ha. While extreme injury was observed in this treatment, the dry weight production was reduced only 5.5% from that of the control. The correlation between percent fresh weight and percent dry weight decreased as the rate of ethalfluralin and degree of visible injury increased. The correlation between the phytotoxicity ratings and percent fresh weight production increased with increasing rates of ethalfluralin and increasing visible injury to the seedlings. This study shows that dry weight production may be a poor indicator of ethalfluralin injury to bean seedlings and that fresh weight production is not as sensitive an indicator of injury as are visible ratings. This study demonstrates that a significant difference in tolerance to higher rates of ethalfluralin exists between <u>Phaseolus</u> <u>vulgaris</u>

Table 3. Correlation coefficients for percent fresh weight and phytotoxicity rating, percent dry weight and phytotoxicity rating and for percent dry weight and percent fresh weight of seven bean cultivars treated with ethalfluralin at 1.12, 2.24, 4.48 or 8.96 kg/ha.[a]

		Variable[b]	
Rate (kg/ha)	Variable	Phytotoxicity Rating	% Fresh Wt.
		(Correlation	Coefficients)
1.12	% Fresh Wt.	-0.070	
	% Dry Wt.	-0.143	0.892**
2.24	% Fresh Wt.	-0.420	
	% Dry Wt.	-0.360	0.849*
4.48	% Fresh Wt.	-0.471	
	% Dry Wt.	-0.138	0.788*
8.96	% Fresh Wt.	-0.709	
	% Dry Wt.	-0.126	0.627

[a]Correlation coefficients are calculated using data for two experiments.

[b]The % fresh and % dry weights are shoot weights expressed as % of untreated control.

Significant at P<0.05(*) or P<0.01(**).

cultivars. Although all cultivars tested showed good tolerance to ethalfluralin at commercially used rates, increased sensitivity of 'Spartan Arrow' could be a concern under adverse growing conditions.

Differential cucumber cultivar tolerance study. As was noted for beans, a reduction in plant height, mass or general plant vigor was also observed in cucumbers treated with preplant incorporated ethalfluralin. There were no significant differences in phytotoxicity ratings among any of the cultivars tested, while all cultivars showed some ethalfluralin toxicity (Table 4). Differences were observed among dry shoot weights of treated cucumber cultivars.

'GP14A', 'Tempo' and 'National Pickling' showed the least reduction, while 'Marketmore 76', 'Marketmore 70' and 'Calypso' had the greatest reduction in fresh weight. 'GP14A' and 'Tempo' showed only slight symptoms of being inhibited by ethalfluralin, while 'Marketmore 70', 'Marketmore 76' and 'Calypso' were substantially inhibited.

The dry weight production of 'GP14A', 'Earlipik' and 'Lemon' was least affected by ethalfluralin, while the 'Marketmore 76', 'Marketmore 70' and 'Calypso' incurred substantial reductions in dry weight production. The correlation coefficient between percent fresh and percent dry weight was 0.803 which was significant at $P<0.01$. The correlation coefficients between phytotoxicity ratings and percent fresh or dry weight for all cultivars were -0.599 and -0.460 respectively, both of which were significant at $P<0.01$. This study shows that cucumbers do not exhibit good tolerance to incorporated ethalfluralin treatments. This study also suggests, based on the

Table 4. Phytotoxicity ratings and influence of ethalfluralin (1.12 kg/ha) on shoot dry weight production as a percent of control.[a]

Cultivar	Rating[b]	Dry Wt. (% of untreated)
A&C Hybrid Improved	3.0	76.6
Lucky Strike	2.6	82.5
Setter	2.5	82.4
Triplemech	2.1	83.3
Bounty	2.0	82.9
Chemset	2.8	76.1
GP14A	1.9	104.8
High Mark II	2.9	84.6
MarketMore 76	3.4	69.6
Poinsett 76	3.8	82.7
Premier	2.5	77.9
Score	3.1	76.4
Spartan Wonder	2.6	84.2
Sprint 440(n)	2.1	80.0
Sprint 440(s)	2.6	80.9
Tamor	3.0	86.1
XP 1191	3.0	82.0
XP 1304	2.8	82.1
XP 1338	2.6	84.0
E 0210	2.8	78.1
E 0212	2.9	89.6
FM 4285	2.5	85.0
FX 4320	2.8	82.1
FX 4551	2.5	81.5
Gemini	2.9	81.9
Green Star	2.4	83.0
Marketmore 70	3.4	72.8
Pennant	2.9	85.4
Raider	2.8	79.2
Regal	2.2	86.9
Slicemaster	2.2	76.5
Tempo	2.1	98.0
Karmon	2.4	87.7
RS 78003	2.6	77.4
RS 78053	2.2	81.3

(continued)

Table 4. (continued)

Cultivar	Rating[b]	Dry Wt. (% of untreated)
RS 79031	2.9	76.7
RS 80022	2.0	83.1
County Fair	2.9	81.8
Double Yield Pickling	1.8	80.3
Lemon	1.9	93.5
Liberty	2.1	86.3
National Pickling	2.5	90.1
Patio Pick	2.2	84.1
Peppi	2.4	86.0
Saladin	2.2	77.7
Salty	2.2	88.7
Spartan Dawn	2.1	86.2
Wisconsin S.M.R. 58	2.5	84.3
Calypso	3.4	72.1
Carolina	3.1	84.4
Earlipik 14	1.9	94.3
Green Spear 14	2.2	86.7
Pikmaster (808)	3.0	80.2
Tri Spear	2.2	79.7
LSD (0.05)	NS	11.8

[a]The phytotoxicity ratings and average shoot weights, taken 16 days after planting, are averaged over two experiments.

[b]Cucumber phytotoxicity ratings were on a 0-10 scale where 0= no effect on height, mass or vigor and 10= complete death of plants.

percent fresh and dry weight data, that differential cucumber tolerances to ethalfluralin occur.

These studies demonstrate that ethalfluralin induces morphological abnormalities similar to those reported for other dinitroaniline herbicides such as root tip swelling, hypocotyl swelling and inhibition of hypocotyl unhooking in beans and inhibition of lateral root development and hypocotyl swelling in cucumber. Inhibition of germination by ethalfluralin was not observed in either beans or cucumbers. All bean cultivars examined in the cultivar tolerance study showed good tolerance to ethalfluralin at practical field rates (1.12 to 2.24 kg/ha) under the conditions of this study. However, at higher treatment rates the 'Spartan Arrow' and 'Seafarer' were severly injured while 'Domino', 'Swan Valley' and 'Montcalm' continued to exhibit good crop tolerance. Under adverse growing conditions, 'Spartan Arrow' and 'Seafarer' might be expected to incur herbicide injury from ethalfluralin at 2.24 kg/ha. Although no significant differences were found in the phytotoxicity ratings for cucumbers treated at 1.12 kg/ha ethalfluralin, significant differences among cultivars were noted in both percent fresh and dry weight. The fresh and dry weights of 'GP14A' and 'Tempo' were not reduced while 'Marketmore 70', 'Marketmore 76' and 'Calypso' had lowered fresh and dry weights. It is not clear from this study whether 'GP14A' and 'Tempo' could tolerate preplant incorporated ethalfluralin treatments under field conditions. These studies show that differential cultivar tolerance to ethalfluralin occurs in both beans and cucumbers. Based on these studies, it appears that bean and cucumber crop tolerance to ethalfluralin has a genetic basis and may well be subject to improvement by selective breeding.

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Chapter 4

ABSORPTION AND TRANSLOCATION OF ^{14}C-ETHALFLURALIN IN CUCUMBER (*Cucumis sativus* L. cv Green Star), BEAN (*Phaseolus vulgaris* L. cv Seafarer) AND PEA (*Pisum sativum* L. cv Sparkle)

ABSTRACT

Field and greenhouse studies were initiated to determine absorption and translocation characteristics of ^{14}C-ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) in vegetable crops. In field studies, cucumbers treated with 1.68 or 3.36 kg/ha preemergence surface applications of ^{14}C-ethalfluralin accumulated significantly more ^{14}C in leaves than in petioles, stems or fruits. Beans and peas treated with preplant incorporated ^{14}C-ethalfluralin were found to contain ^{14}C in the plant parts tested. In greenhouse studies, ^{14}C-ethalfluralin vapor was found to be absorbed by both cucumber seedling stems and cotyledon tissue. Both bean and cucumber seedlings were found to absorb ^{14}C-ethalfluralin from a nutrient solution and translocated ^{14}C into all plant parts. Both cucumber and bean absorbed ^{14}C-ethalfluralin topically applied to the stem and translocated ^{14}C acropetally while only limited basipital translocation occurred. Although both cucumber and bean cotyledons absorbed ^{14}C-ethalfluralin, very little ^{14}C moved out of the treated tissue.

INTRODUCTION

Few herbicides are available which selectively control broad-leaved weeds and grasses in cucurbit crops. This is because cucurbits are sensitive crops and exhibit only moderate tolerance to the few herbicides currently registered. Recent work has demonstrated good weed control in cucurbit crops with the dinitroaniline herbicide ethalfluralin (11,12,18). However, crop tolerance and mechanism of selectivity in cucurbit crops to ethalfluralin are not well understood.

Parker (10) demonstrated that chlorpropham (isopropyl m-chlorocarbanilate), dichlobenil (2,6-dichlorobenzonitrile) and trifluralin (α,α,α,-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) can exert their herbicidal action through direct uptake by the shoot of Sudax (Sorghum vulgare x sudanense), but root uptake appeared to be much more effective. Barrentine and Warren (1) reported that nitralin (4-(methylsulfonyl)-2,6-dintro-N,N-dipropylaniline) was more toxic to cucumber roots than trifluralin. However, trifluralin applied to the cucumber shoot zone was more effective in reducing shoot weight than was nitralin. In another study (2), they reported that more ^{14}C-trifluralin and ^{14}C-nitralin were taken up in the hypocotyl hook region of cucumber seedlings than from cotyledons or the hypocotyl region below the hypocotyl hook. Derr and Monaco (3) found that both root and shoot uptake of ethalfluralin occurs in cucumbers, but that shoot exposure was less injurious to cucumber growth than root exposure.

The relative toxicities of dinitroaniline herbicides vary widely and are species dependent (1,8). Jordan et al. (8) reported that the relative vapor toxicities of several dinitroaniline herbicides to Japanese millet (Echinochloa crus-galli (Roxb.) Wight) generally correlated with their vapor pressures. One exception noted by Jordan was that dinitramine (N^4,N^4diethyl-α,α,α-trifluoro-3,5-dinitrotoluene-2,4-diamine) though not as volatile as other compounds, was highly toxic to Japanese millet shoots. Other investigators have also confirmed vapor activity of dinitroaniline herbicides (4,6,9,14,16). Savage (13) found that ethalfluralin volatility from soil approximated that of trifluralin. Since the entire plant may be exposed to these volatile herbicides through vapor contact, each plant part is a possible site of herbicide uptake.

Only limited translocation of dinitroaniline herbicides within plants has been reported to occur. Little ^{14}C-oryzalin (3,5-dinitro-N^4,N^4-dipropyl-sulfanilamide) was found to move from corn (Zea mays L.) roots to shoots (17). Jacques and Harvey (7) observed some root to shoot translocation to benefin (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine), dinitramine, fluchloralin (N,(chloroethyl)-2,6-dinitro-N-propyl-4-(trifluoro methyl)aniline), profluralin (N-(cyclopropyl methyl)-α,α,α-trifluoro-2,6-dinitro-N-propyl-p-toluidine) and trifluralin in peas (Pisum sativum L.), but no shoot to root transport was detected. In a microradioautographic study, Strang and Rogers (15) found that radioactivity from ^{14}C-trifluralin was retained primarily on the surface of the treated cotton (Gossypium hirsutum L.) and soybean (Glycine max (L.) Merr.) roots. Radioactivity was noted in the walls of xylem vessels and cortical cells in roots of both cotton and soybeans. Radioactivity was also noted in the protoxylem of the cotton stem.

The purpose of this study was to assess the degree of ethalfluralin uptake by various parts of cucumber and bean plants, the subsequent translocation of the absorbed herbicide, and to determine if these factors influence selectivity in these crops.

METHODS AND MATERIALS

Field Study

Cultural practices. A field site was selected at the Michigan State University Horticultural Research Farm, East Lansing, Michigan on a Spinks loamy sand soil (Appendix Table A1). Four galvanized metal culverts 914 cm in diameter and 610 cm in length were placed vertically into the soil a maximum depth of 457 cm with a minimum disturbance of the naturally occurring soil profiles. Uniformly ring labeled ^{14}C-ethalfluralin with a specific activity of 2.78 μci/μmole was amended with technical grade (98.6%) ethalfluralin and dissolved in acetone to produce the following ^{14}C-ethalfluralin solutions: (A) a 250 ml solution of 110.4 mg (0.753 μci/μmole), (B) a 250 ml solution of 220.9 mg (0.376 μci/μmole), (C) a 50 ml solution of 110.4 mg (0.753 μci/μmole), and (D) a 50 ml solution of 220.9 mg (0.376 μci/μmole) ethalfluralin. Analysis by TLC indicated the purity of the ^{14}C-ethalfluralin to be greater than 97%.

Cucumber. Cucumber seeds were planted at a depth of 2.5 cm in each of two culverts on July 17, 1980. The ^{14}C-ethalfluralin solutions C and D were each throughly mixed with 900 cm^3 soil samples which were removed from the seeded culverts. Each soil sample, after treating, was evenly distributed over the soil surface within the individual culverts on July 18, 1980. These two treatments resulted in ^{14}C-ethalfluralin being applied at rates of 1.68 and 3.36 kg/ha respectively. Immediately after the treated soil was applied to the sites, each culvert was irrigated with approximately 1.0 cm of water. The individual sites were hand irrigated as required throughout the growing season. Plants in both treatments were harvested at soil level 56 days after treatment. Plants were throughly cleaned of surface soil by rinsing with water and scrubbing with a brush as required. Individual plants were seperated into leaves, petioles, stems and fruit, placed into plastic bags, frozen and stored at -25 C.

Beans and peas. On July 17, 1980, the top 6.4 cm layer of soil was removed from an untreated culvert and placed into a metal drum equipped with a tight fitting cover. Solution A was throughly mixed with the soil in the drum by rolling the drum. The treated soil was replaced into the culvert and evenly distributed. Solution B was applied to the soil from a second culvert in a similar manner. These two treatments resulted in ^{14}C-ethalfluralin being applied at rates of 1.68 and 3.36 kg/ha respectively. Beans and peas were planted at a depth of 2.5 cm in each culvert and then irrigated with approximately 1.0 cm of water. The individual sites were sprinkle irrigated to prevent stress throughout the growing season.

Pea plants in both the 1.68 and 3.36 kg/ha treatments were harvested at soil level 49 days after treatment. Plants were cleaned of surface soil by rinsing with water. Plants were separated into leaves, stems, pods and seeds with each type of plant part being frozen, partially lyophilized and allowed to dry to air dryness at room temperature.

Bean plants in the 1.68 kg/ha treatments were harvested both two and four weeks after treatment, throughly cleaned of surface soil, and prepared for autoradiography. Mature bean plants in both the 1.68 and 3.36 kg/ha treatments were harvested at soil level 98 days after treatment. Plants were cleaned of surface soil by scrubbing with a brush. Pods and seeds were seperated from stem material and dried for radioassay.

Radioassay and Statistical Analysis. Ten cucumber fruits from both the 1.68 and 3.36 kg/ha treatments were individually homogenized in a Sorvall Omni-Mixer at high speed. Plant homogenates were evenly slurried into aluminum pans and frozen on dry ice. For each fruit sample, 10 sub-samples of the frozen homogenate were taken for combustion. For leaves, stems and petioles, five sub-samples were taken from each of five plants for combustion. Representative bean and pea samples were also taken from the collected plant material for combustion.

Samples (300 mg) were combusted in an OX-200 Harvey Biological Oxider at 900 C with an O_2 flow rate of 300 cm^3/min for 4 min. $^{14}CO_2$ was absorbed in 15 ml 2:1 (v/v) Permaflour V liquid scintillation counting solution: Carbosorb II CO_2 absorbant (Packard) and radioassayed by liquid scintillation spectrometry. Each sample was counted for 100 min or 4,000 counts, whichever occurred first. Counts for each sample were corrected for quenching by external standard channels ratio, background and oxidation efficiency. The disintegrations per min (DPM) for each sample and sub-sample was converted to DPM/g basis and the average DPM/g for each sample was calculated. All quantitative data for ^{14}C-residues were calculated as ^{14}C-ethalfluralin equivalents. Data were subjected to ANOV and plant part comparisons were achieved with paired t-tests.

Greenhouse Studies

Vapor uptake study. Individual cucumber seedlings were germinated in styrofoam cups containing a greenhouse soil mix (1:1:1, v/v/v, sand: peat:soil). One day after the cotyledons were fully expanded, a glass cover slip with 228,000 DPM (2.78 μci/μmole) ^{14}C-ethalfluralin was positioned horizontally 1 cm below the cotyledon and 0.5 cm from the stem. The plants were maintained in a greenhouse at 28±4 C day and 20±4 C night with 50-70% R.H. for 24 h. Supplemental lighting was provided by metal halide lamps with a 16 h photo period.

At 6 and 24 h after treatment, plants were harvested by washing soil from the root system and either dividing the plants into roots, stem, adjacent and opposite cotyledons for combustion or prepared for autoradiography. Samples were combusted as described previously and the ^{14}C quantitated for each plant part. The ^{14}C-residue remaining on the cover slip was determined by placing the cover slip in a scintillation vial with 15 ml of ACS (Amersham), a prepared liquid scintillation cocktail, and quantitating by liquid scintillation spectrometry. Four replications of one plant for each treatment were employed. The percent ^{14}C-ethalfluralin volitilized, percent ^{14}C-vapor absorbed and distribution of ^{14}C in root, stem, untreated cotyledon and treated cotyledon was calculated. All data represent the means of two experiments.

Root uptake study. Bean and cucumber seeds were germinated in vermiculite moistened with one-half strength Hoagland's solution (5). Two days after the unhooking of the hypocotyls, the vermiculite was washed from the plant roots. Two bean or cucumber plants were suspended in a 100 ml glass beaker covered with aluminum foil and containing 60 ml of one-half strength Hoagland's solution (5). The beakers were aerated by bubbling air through the nutrient solution for 1 min every 30 min and the nutrient solution was replenished as needed throughout the experiment. After an acclimation period of 24 h, a 3 μl aliquot of an acetone solution containing 666,000 DPM of ^{14}C-ethalfluralin (2.78 μci/μmole) was added to the nutrient solution of each beaker. A 1 cm layer of activated charcoal was then placed on top of the foam disk used to suspend the plants to adsorb volatilized ^{14}C-ethalfluralin. This study was maintained in a greenhouse under conditions previously described.

Plants were harvested 2, 6, 24 or 48 h after treatment by removing the plants from the treated nutrient solution and agitating the roots in 100 ml of one-half strength Hoagland's solution for 1 min. The plants were either prepared for autoradiography or divided into roots, stems, cotyledons and leaves all of which were stored at -26 C for analysis. The plant samples were combusted as previously described and the ^{14}C quantitated by plant part. Three replications of two plants for each treatment were employed. The total ^{14}C absorbed by each plant and the percent distribution of ^{14}C in each plant part was calculated. All data are presented as the means of two experiments.

Stem and cotyledon uptake studies. Beans and cucumber seeds were germinated in styrofoam cups containing a greenhouse soil mix (previously described). Two days after hypocotyl unhooking a 3 μl aliquot of methanol containing 252,000 DPM of ^{14}C-ethalfluralin (2.78 μci/μmole) was applied in a 1 cm band around the seedling stem in the region 1 cm to 2 cm below the cotyledons. The surface area treated was 1 cm^2 on the the bean stem and 0.6 cm^2 on the cucumber stem. The cotyledons of another series of plants were treated one day after hypocotyl unhooking with 275,000 DPM of ^{14}C-ethalfluralin (2.78 μci/μmole) applied evenly over the lower surface of one bean cotyledon or over the end 1 cm length of the upper surface of one cucumber cotyledon. The treated bean and cucumber cotyledon surface areas were ca 1 and 0.8 cm^2 respectively. The study was maintained in a greenhouse under conditions previously described.

Plants receiving stem applications of ^{14}C-ethalfluralin were harvested at soil level 2, 4, 6 and 8 h after treatment and prepared for microautoradiography. Additional plants receiving stem treatments and plants receiving cotyledon treatments were harvested 6, 24 and 96 h after treatment by washing the soil from the roots with water and rinsing the treated area with 20 ml methanol. The plants were either prepared for autoradiography or divided into roots, stem, cotyledon and leaves which were stored at -26 C until combusted. The plant samples were combusted as previously described and the ^{14}C quantitated by plant part. Four replications of one plant for each treatment were employed. The total ^{14}C absorbed by each plant and the percent distribution of ^{14}C in each plant part was calculated. All data presented are the means of two experiments.

^{14}C-Extraction and Characterization Study. Cucumbers and beans were germinated, treated, harvested and stored as described for the root uptake study. Roots, stems and cotyledons from two plants at each exposure time were homogenized and extracted with 80 % methanol (CH_3OH) (20 ml). The extract was filtered through 540 Whatman paper and the methanol removed by evaporation under vacuum at 35 C. The aqueous extract was partitioned with methylene chloride (CH_2Cl_2) (4 X 10 ml). The pooled methylene chloride phase was reduced in volume under vacuum at 35 C to 1.0 ml. The aqueous fraction and a 0.1 ml aliquot of the methylene chloride fraction were individually assayed for radioactivity by liquid scintillation spectrometry using 15 ml ACS (Amersham) scintillation solution. The filter paper and methanol insoluble residue was combusted and radioassayed as described previously.

A single study consisting of two plants for each treatment was employed. The total ^{14}C absorbed by each set of plants and the percent distribution of ^{14}C in each fraction for each part was determined.

RESULTS AND DISCUSSION

Field Study

Cucumbers. More ^{14}C accumulated in cucumber leaves at both treatment rates than in petioles, stems and fruits which all contained similar levels of ^{14}C (Table 1). Leaves and petioles contained 1.8 to 2.4 fold more ^{14}C in the 3.36 kg/ha than the 1.68 kg/ha

Table 1. ^{14}C-residue in cucumber tissue following preemergence soil applications of ^{14}C-ethalfluralin at 1.68 and 3.36 kg/ha.

Plant Part	Mean ^{14}C-residue Rate (kg/ha) 1.68	3.36
	(PPM)	
Leaf	.164a	.298a*
Petiole	.017b	.041b*
Stem	.026b	.041b
Fruit	.017b	.017b

Mean ^{14}C-residue calculated on fresh weight of plant material.

Means within a column followed by the same letter are not significantly different ($P<0.05$, t-test).

*Significantly different from 1.68 kg/ha treatment ($P<0.05$, t-test).

treatment while ^{14}C detected in both stems and fruits were similar to each other at both treatment rates. Cucumber leaves appear to be a sink for absorbed ^{14}C-ethalfluralin or metabolites and fruit, though actively growing, do not accumulate appreciable amounts of ^{14}C. ^{14}C accumulated in both leaves and petioles approximately doubled with a 2 fold increase in ^{14}C-ethalfluralin application.

Beans and peas. At both rates of ^{14}C-ethalfluralin, 3 to 4 times as much ^{14}C accumulated in bean stems than pods (Table 2). Significantly less ^{14}C was found in seeds than either pods or stems. Stems, pods and seeds in the 3.36 kg/ha treatment contained more than twice as much ^{14}C than the comparable plant part in the 1.68 kg/ha treatment. The autoradiograph of a bean plant after 2 weeks soil exposure to ^{14}C-ethalfluralin shows ^{14}C is distributed in all plant parts with the highest concentrations being in the roots and vascular system (Figure 1). A comparable autoradiograph after 4 weeks soil exposure shows ^{14}C is distributed in new leaf and stem tissue while ^{14}C also continues to persist in the vascular system of the oldest leaves (Figure 2). It appears that ^{14}C-ethalfluralin is absorbed by bean plants and parent compound and/or metabolites are translocated to the leaves and largely immobilized in the vascular system.

The 3.36 kg/ha ^{14}C-ethalfluralin treatment was extremely phytotoxic to peas. Only 10% of the pea seedlings emerged, while those that did emerge were severely stunted compared to untreated guard plants. Although pea emergence was not inhibited in the 1.68 kg/ha treatment, plants were noticeably stunted. Leaves from peas treated with 1.68 kg/ha ^{14}C-ethalfluralin contained nearly twice as much

Table 2. ^{14}C-residue in beans and peas following pre-plant incorporated treatments of ^{14}C-ethalfluralin at 1.68 and 3.36 kg/ha.[a]

Plant	Beans		Peas	
	Rate (kg/ha)		Rate (kg/ha)	
Part	1.68	3.36	1.68	3.36
	No. Samples/mean			
Leaf	-	-	6	5
Stem	9	9	6	5
Pod	20	20	6	-
Seed	20	20	6	-
	Mean ^{14}C-residue (PPM)[b]			
Leaf	-	-	3.958a	7.151a
Stem	3.306a	10.420a	2.157b	6.443a
Pod	1.053b	2.749b	0.383c	-
Seed	0.053c	0.126c	0.142c	-

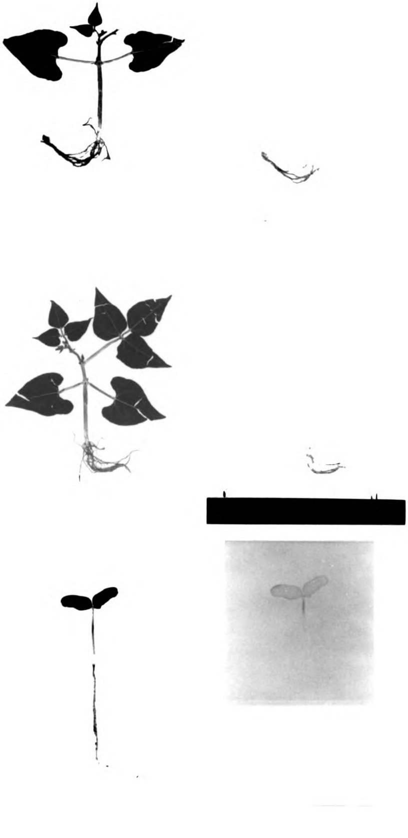
[a]Mean ^{14}C-residue calculated on air dry weight of beans and fresh weight of peas.

[b]Means within a column followed by the same letter are not significantly different; means are significantly different between rates for each plant part tested ($P<0.05$, t-test).

Figure 1. Distribution of radioactivity in a bean plant 2 weeks after treatment with soil applied ^{14}C-ethalfluralin. The plant specimen is on the left and the autoradiograph on the right.

Figure 2. Distribution of radioactivity in a bean plant 4 weeks after treatment with soil applied ^{14}C-ethalfluralin. The plant specimen is on the left and the autoradiograph on the right.

Figure 3. Distribution of radioactivity in a cucumber seedling after 6 hours exposure to ^{14}C-ethalfluralin vapor. The cotyledon adjacent to treated glass plate is on the left. The plant specimen is on the left and the autoradiograph on the right.



^{14}C than stems from the same treatment. The ^{14}C in pods and seeds were similar to each other but 5 to 15 times less than stem ^{14}C levels. Leaves and stems of peas in the 3.36 kg/ha treatment contained more ^{14}C than the same plant parts from the 1.68 kg/ha treatment but were not different from each other.

Greenhouse Studies

Vapor uptake study. ^{14}C-ethalfluralin readily volatilized from the surface of the glass disc with 81.1% of the applied material being volatilized after 24 h (Table 3). The total ^{14}C-ethalfluralin absorbed by the cucumber seedlings was 0.4 and 0.6% after 6 and 24 h exposures respectively. The autoradiograph of a seedling after 6 h exposure shows that ^{14}C-vapor was absorbed by the stem region adjacent to and above the level of the glass disc (Figure 3). Although significantly more ^{14}C-ethalfluralin volatilized after 24 h than 6 h the percent ^{14}C detected in the adjacent cotyledons and stems did not differ from that found after 6 h exposure. The adjacent cotyledons contained slightly more ^{14}C than the opposite cotyledons after 6 h exposure, but this difference did not persist until 24 h after exposure. More ^{14}C was detected in the opposite cotyledons after 24 h exposure as compared to 6 h exposure. The data suggests mobility of ^{14}C from the treated cotyledon to the untreated cotyledon as well as acropetal movement of ^{14}C from the stem to the untreated cotyledon. More ^{14}C was observed in roots after 24 h than at 6 h which suggests basipital translocation of ^{14}C-ethalfluralin does occur to a limited degree.

Root uptake study. The percent of absorbed ^{14}C detected in cucumber roots decreased while the ^{14}C observed in cotyledons increased at each sampling period (Table 4). The autoradiograph of a cucumber

Table 3. Volatilization and absorption of ^{14}C-ethalfluralin by cucumber seedlings.[a]

^{14}C Distribution	Exposure Time 6 h	Exposure Time 24 h
	%	%
Volatilized	74.6	81.1*
Vapor absorbed	0.4	0.6
	% of absorbed	% of absorbed
Adjacent cotyledon	43.5	40.2
Opposite cotyledon	29.2	38.6*
Stem	27.2	20.7
Root	0.1	0.5*
LSD (0.05)	8.0	9.1

[a]Means are average for two experiments.

*Significantly different from 6 h treatment ($P<0.05$, t-test).

Table 4. Distribution of ^{14}C in cucumber and bean seedlings following exposure of roots to ^{14}C-ethalfluralin.[a]

Plant	Exposure Time	^{14}C Distribution Root	Stem	Cotyledon	Leaf	Total ^{14}C absorbed
	(h)	%				(DPM/Plant)
Cucumber	2	90.63	6.15	3.22	-	17921
	6	82.85	10.93	6.22	-	13362
	24	76.38	10.12	13.48	-	11585
	48	67.52	11.58	20.87	-	14413
	LSD(0.05)	4.42	2.68	2.90		N.S.
Bean	2	96.85	2.63	0.00	0.48	41492
	6	89.20	7.13	0.08	3.58	28550
	24	80.27	11.72	0.33	7.67	22863
	48	79.72	9.45	0.37	10.43	28047
	LSD(0.05)	3.08	1.90	0.16	1.72	7418

[a]Means are average for two experiments.

seedling after 24 h root exposure to ^{14}C-ethalfluralin indicate that the ^{14}C in the cotyledons is concentrated in the vascular system (Figure 5A). A similar autoradiograph of a bean seedling also shows a concentration of ^{14}C in the vascular system of its leaves (Figure 4A). The percent ^{14}C found in cucumber stems remained relatively constant after 6 h.

The percent ^{14}C observed in bean roots decreased at the 6 h and 24 h exposure intervals after which it remained similar until the 48 h period. The percent ^{14}C in bean stems increased with each exposure period until 24 h exposure, after which the level decreased slightly. Only .37% of the total absorbed ^{14}C was observed in the bean cotyledons 48 h after root exposure. The percent ^{14}C detected in bean leaves increased at each exposure time until at 48 h, 10.43% of the total absorbed ^{14}C was observed in the leaves.

These data show that ^{14}C-ethalfluralin is absorbed by the roots of cucumber and bean seedlings and that substantial ^{14}C-material is translocated to the cotyledons of cucumbers and the leaves of beans. The autoradiographs indicate that ethalfluralin or metabolites move readily in the vascular system of the plants.

<u>Stem and cotyledon uptake study.</u> Less than 1% of the total ^{14}C-ethalfluralin absorbed was translocated to the roots of either cucumber or bean seedlings after stem exposure (Table 5). After 96 h exposure, 40.04% of the absorbed ^{14}C-material was detected in the cotyledons of cucumber seedlings. In contrast, bean cotyledons contained only 4.25% and leaves 12.02% of the absorbed ^{14}C. Absorption and translocation of ^{14}C-material was much more rapid in cucumbers than in beans after stem

Figure 4. Distribution of radioactivity in bean seedlings 24 h after treatment with ^{14}C-ethalfluralin to (A) the root system, (B) the stem and (C) a single cotyledon. The plant specimen is on the left and the corresponding autoradiograph on the right.

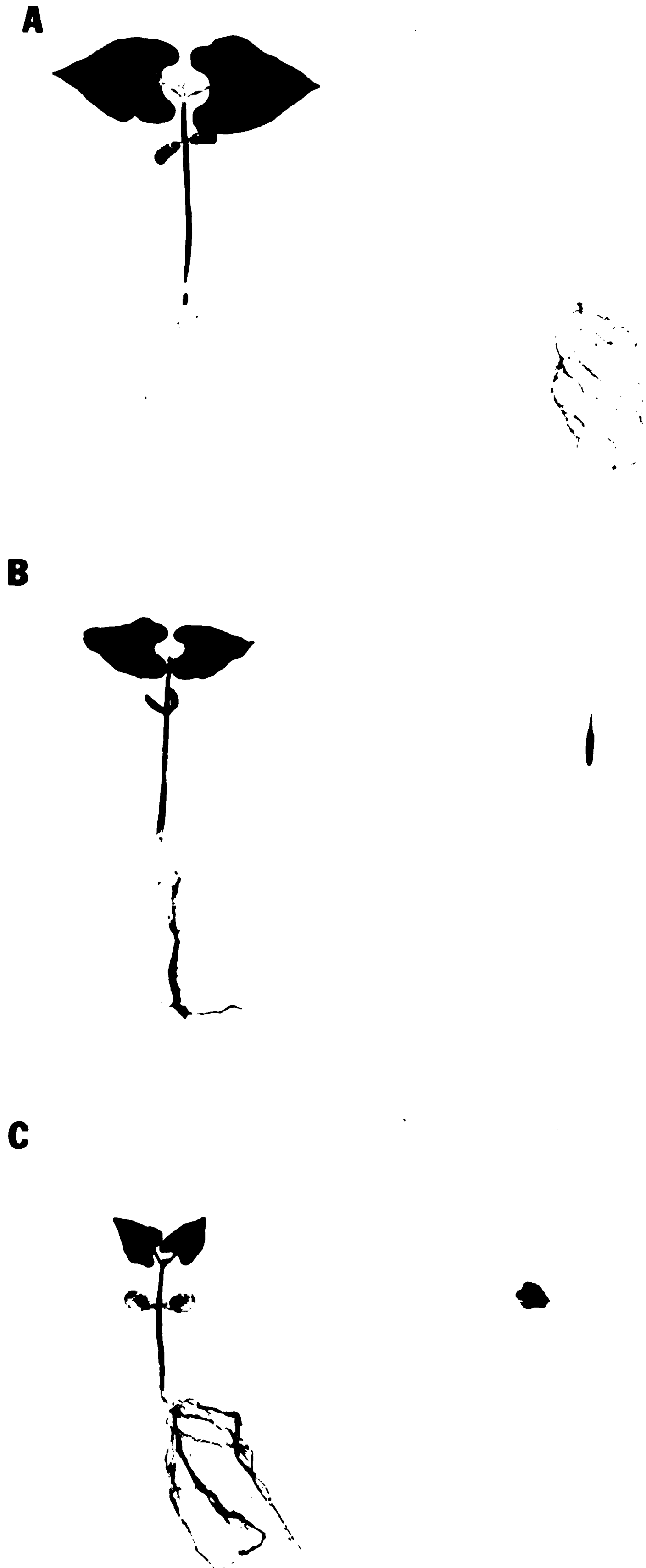

A
B
C

Figure 5. Distribution of radioactivity in cucumber seedlings 24 h after treatment with ^{14}C-ethalfluralin to (A) the root system, (B) the stem and (C) a single cotyledon. The plant specimen is on the left and the corresponding autoradiograph on the right.

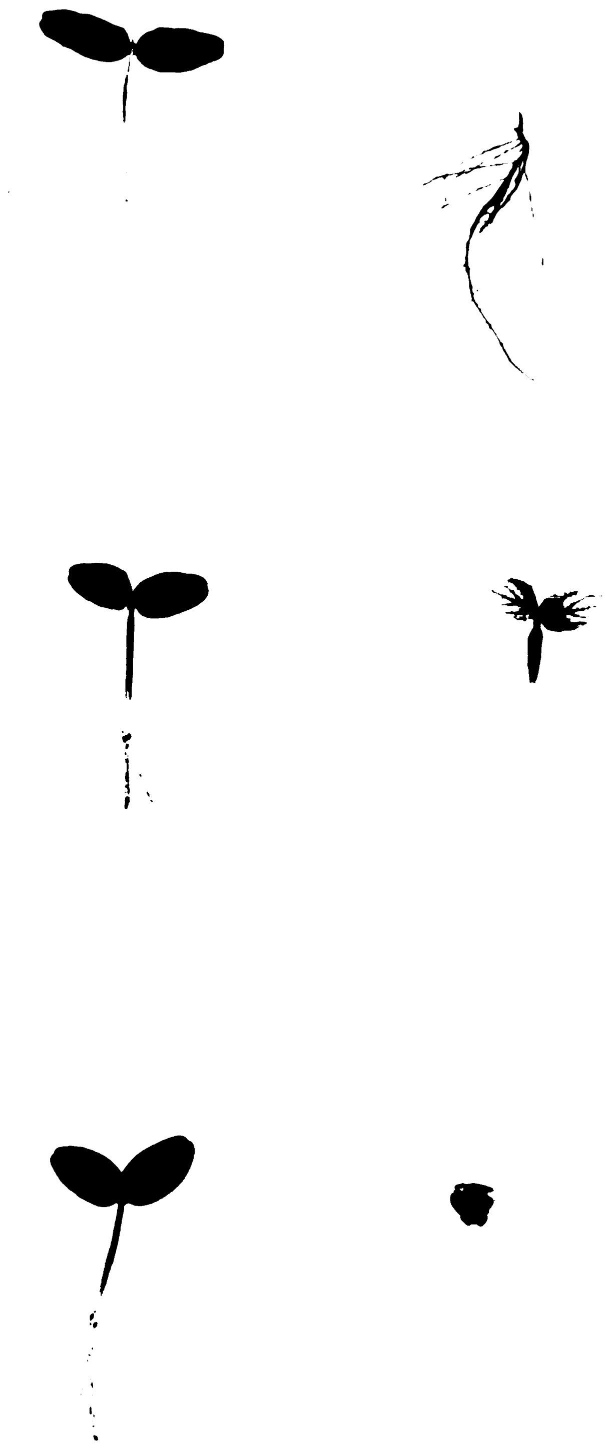


Table 5. Distribution of ^{14}C in cucumber and bean seedlings following stem exposure to ^{14}C-ethalfluralin.[a]

Plant	Exposure Time	^{14}C Distribution Root	Stem	Cotyledon	Leaf	Total ^{14}C absorbed
	(h)	%				(DPM/Plant)
Cucumber	6	.34	83.60	16.05	-	27786
	24	.85	63.71	35.45	-	29280
	96	.78	59.19	40.04	-	30947
	LSD(0.05)	N.S.	5.53	5.76		N.S.
Bean	6	.10	96.64	1.46	1.80	14871
	24	.35	89.46	2.45	7.74	22313
	96	.81	82.91	4.25	12.02	33402
	LSD(0.05)	.17	1.34	1.07	1.12	5447

[a]Means are average for two experiments.

exposure to ^{14}C-ethalfluralin. Though a portion of the ^{14}C found in the cucumber cotyledons and bean leaves may have resulted from vapor absorption, the autoradiographs of cucumbers and beans receiving stem treatments show a concentration of ^{14}C in the vascular system indicating translocation of ^{14}C (Figure 4B and 5B). Microradiographs of cucumber stem tissue from the region above the site of ^{14}C-ethalfluralin application shows ^{14}C-residues to be prominent in xylem tissue 6 h post treatment (Figure 6A and 6B). Similarly, ^{14}C-residue was observed in xylem tissue below the site of application though not as concentrated (Figure 6C). In a cucumber leaf cross-section, ^{14}C-residue was again found to be concentrated in xylem tissue (Figure 6D). ^{14}C-residue was concentrated in the intercellular spaces near the epidermis and in xylem tissue of bean stem cross-sections taken above the application site at 8 h post treatment (Figure 6E and 6F).

After a 96 h exposure of cotyledons to ^{14}C-ethalfluralin, 97.5% of the absorbed ^{14}C was located in the treated cucumber cotyledons (Table 6). Both the data and the autoradiographs indicate that very little movement of ^{14}C occurred out of treated cucumber cotyledons (Figure 5C). The ^{14}C absorbed by bean cotyledons was somewhat more mobile with some movement evident into the stem and leaves (Figure 4C).

^{14}C-Extraction and Characterization Study. The percent absorbed ^{14}C detected in the methanol insoluble fraction increased in both cucumber and bean with exposure time (Appendix Table A2 and A3 and Table 7). The percent ^{14}C observed in the methylene chloride fraction of bean extract was maximum (82.0 %) after 2 h exposure then decreased with time. The percent ^{14}C in the methylene chloride fraction of

Figure 6. Microradiographs of cucumber and bean seedlings following topical stem applications of ^{14}C-ethalfluralin: (A) cucumber stem above application zone 6 h post treatment (X 300); (B) cucumber stem vascular tissue above application zone 6 h post treatment (X 300); (C) cucumber stem vascular tissue below application zone 6 h post treatment (X 875); (D) cucumber leaf vascular tissue 8 h post treatment (X 300); (E) bean stem tissue near epidermis above treatment zone 8 h post treatment (X 300); (F) bean stem vascular tissue above treatment zone 8 h post treatment (X 300).

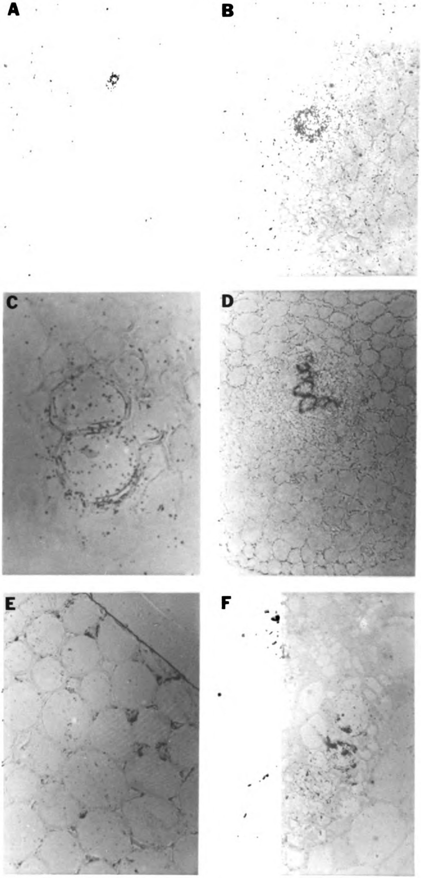

A
B
C
D
E
F

Table 6. Distribution of ^{14}C in cucumber and bean seedlings following cotyledon exposure to ^{14}C-ethalfluralin.[a]

Plant	Exposure Time	Root	Stem	Untreated Cotyledon	Treated Cotyledon	Leaf	Total ^{14}C Absorbed
		^{14}C Distribution					
	(h)	%					(DPM/Plant)
Cucumber	6	.09	.88	2.85	96.24	-	19370
	24	.10	.35	2.24	97.30	-	29087
	96	.21	.36	1.76	97.50	.16	44024
	LSD(0.05)	.04	N.S.	N.S.	N.S.		6998
Bean	6	.02	1.85	1.00	93.76	3.36	18470
	24	.02	1.14	0.52	96.88	1.44	39418
	96	.03	2.04	0.88	94.20	2.55	44369
	LSD(0.05)	N.S.	N.S.	N.S.	1.90	N.S.	14052

[a]Means are average for two experiments.

Table 7. Extraction and phase separation of radioactivity in cucumbers and beans after 2, 6, 24 or 48 hr root exposure to C-ethalfluralin.

Exposure Time	Plant	Recovered Radioactivity: CH_2Cl_2 Phase	Water Phase	CH_3OH Insoluble
		(% of Total)		
2 h	Cucumber	52.4	43.1	4.5
	Bean	82.0	10.3	7.7
6 h	Cucumber	53.3	33.2	13.5
	Bean	53.6	23.8	22.6
24 h	Cucumber	31.1	31.3	37.6
	Bean	25.1	28.2	46.7
48 h	Cucumber	21.9	34.7	43.4
	Bean	21.5	27.5	51.0

cucumber extract decreased after 6 h exposure. After 2 h, the percentage of ^{14}C detected in the water soluble fraction of cucumber extract was four times greater than bean extract. For each treatment time, a higher percentage of ^{14}C was found in the water soluble fraction of cucumber than bean while the reverse was true of the methanol insoluble fraction.

Cucumbers, beans and peas absorbed ^{14}C-ethalfluralin under field growing conditions and accumulated ^{14}C in both leaf and stem tissue. Neither cucumber fruit nor bean seeds accumulated appreciable amounts of ^{14}C in the field study. Nearly 3 times as much ^{14}C was detected in pea seeds than bean seeds after the 1.68 kg/ha treatment. The low tolerance observed in peas to ethalfluralin may be related in part to increased shoot exposure to ethalfluralin due to the slower hypogeous germination of the peas as compared to the rapid epigeous germination of beans.

The vapor uptake study demonstrated that ^{14}C-ethalfluralin vapor is absorbed by both cucumber stems and cotyledons. The root uptake study confirms that ethalfluralin is readily absorbed from a nutrient solution by both cucumber and bean roots and readily translocated to cotyledonary leaves and leaves respectively. The disappearance of ^{14}C from the roots of both cucumber and beans in the root uptake studies was possibly due to transport of ^{14}C-material out of the root system or a sorption-desorption process between the roots and the nutrient solution. Root absorbed ^{14}C-ethalfluralin was rapidly transformed into a water soluble compound in both cucumber and bean. This conversion to a water soluble material most likely preceded the xylem loading and extensive translocation previously described. In both cucumber and bean, the ^{14}C-residue was gradually converted into a methanol insoluble form. It is reasonable to suspect that ethalfluralin vapors in the soil would also be absorbed by cucumber and bean roots. Stems of cucumber and bean seedlings did absorb topically applied ethalfluralin and subsequently translocated the absorbed ^{14}C acropetally into the leaves. Both cucumbers and beans absorbed ^{14}C-ethalfluralin applied to the cotyledons but little translocation of the absorbed material occurred.

Since cotyledons, stems and roots are sites of ethalfluralin absorption, any condition which impedes growth of these plant parts out of the soil herbicide zone may result in subsequent herbicide injury.

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APPENDIX

Appendix

Table A1. Soil Analysis of Spinks Loamy Sand.

Soil PH: 5.7
% O.M. 2.84
CEC: 12.69 MEQ/Gram

Nutrients	Assay	
phosphorus	233	kg/ha
potassium	367	kg/ha
calcium	1912	kg/ha
magnesium	251	kg/ha
nitrate	146.2	PPM
iron	44	PPM
manganese	3	PPM
zinc	3	PPM

Exchangeable bases	Percent of total
potassium	7.5
calcuim	75.9
magnesium	16.6

Table A2. Extraction and phase separation of radioactivity in cucumbers after 2, 6, 24 or 48 h root exposure to ^{14}C-ethalfluralin.

		Recovered Radioactivity		
Exposure Time	Plant Part	CH_2Cl_2 Phase	Water Phase	CH_3OH Insoluble
		(% of Total)		
2 h	Root	50.9	44.7	4.4
	Stem	77.1	16.3	6.6
	Cotyledon	81.9	11.7	6.4
	Total	52.4	43.1	4.5
6 h	Root	48.6	37.1	14.3
	Stem	75.6	13.4	11.0
	Cotyledon	65.6	24.4	10.0
	Total	53.3	33.2	13.5
24 h	Root	22.7	30.1	47.2
	Stem	43.6	36.1	20.2
	Cotyledon	44.9	31.8	23.3
	Total	31.1	31.3	37.6
48 h	Root	15.8	29.8	54.4
	Stem	28.2	45.0	26.8
	Cotyledon	30.0	39.5	30.5
	Total	21.9	34.7	43.4

Table A3. Extraction and phase separation of radioactivity in beans after 2, 6, 24 or 48 h root exposure to ^{14}C-ethalfluralin.

		Recovered Radioactivity		
Exposure Time	Plant Part	Ch_2Cl_2 Phase	Water Phase	Ch_3OH Insoluble
		(% of Total)		
2 h	Root	76.6	12.9	10.5
	Stem	92.6	4.8	2.6
	Cotyledon	63.7	20.3	16.0
	Total	82.0	10.3	7.7
6 h	Root	50.6	25.2	24.2
	Stem	71.9	13.5	14.6
	Cotyledon	56.6	25.8	17.6
	Total	53.6	23.8	22.6
24 h	Root	21.9	27.8	50.3
	Stem	39.4	27.6	33.0
	Cotyledon	39.2	33.7	27.1
	Total	25.1	28.2	46.7
48 h	Root	14.7	28.1	57.2
	Stem	44.9	19.2	35.9
	Cotyledon	30.6	34.8	34.6
	Total	21.5	27.5	51.0

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