# SELECTIVITY OF ETHOFUMESATE (2-ETHOXY-2,3-DIHYDRO-3,3-DIMETHYL-5-BENZOFURANYL METHANESULPHONATE) IN SUGARBEET (BETA VULGARIS L.) AND ASSOCIATED WEED SPECIES

By

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ABSTRACT

SELECTIVITY OF ETHOFUMESATE (2-ETHOXY-2,3-DIHYDRO-3,3-DIMETHYL-5-BENZOFURANYL METHANESULPHONATE) IN SUGARBEET (BETA VULGARIS L.) AND ASSOCIATED WEED SPECIES

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Preemergence application of the combination of ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate) plus TCA (trichloroacetic acid) caused greater growth suppression than pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) plus TCA when followed by a postemergence herbicide treatment to field grown sugarbeets. Sugarbeet tolerance was least when treatments were applied at the early two-leaf stage. Combination treatments applied with endothall [7-oxabicyclo (2,2,1) heptane-2,3-dicarboxylic acid] caused less injury. Foliar treatment at the two- to four-leaf stage of sugarbeet gave greater weed control. Mid-afternoon applications were more selective than either morning or evening treatments.

Exposure of plants to ethofumesate severely decreased the epicuticular wax deposition on the leaf surfaces of sugarbeet. Gas-liquid chromatography indicated ethofumesate decreased the deposition of the alkanes and sec-ketones but increased that of the long-chain waxy esters. Scanning electron micrographs and cuticular transpiration data further

substantiated the inhibition of wax by ethofumesate. Greater absorption of $^{14}$C-ethofumesate, $^{14}$C-desmedipham, and $^{14}$C-ethofumesate + $^{14}$C-desmedipham was observed in plants that received preemergence treatments of ethofumesate and TCA as compared to pyrazon or a control. The basis for the observed interaction of sequential herbicide combinations was increased absorption of foliar applications due to reduction in epicuticular leaf wax deposition from soil-applied herbicides.

Further greenhouse and laboratory studies evaluated the basis for selectivity of both root-applied and foliar-applied ethofumesate on sugarbeet and several weed species. More root-applied $^{14}$C-ethofumesate was translocated to the leaf tissue of the susceptible pigweed and lambsquarter plants than the tolerant sugarbeet and ragweed. Stem tissue of both sugarbeet and ragweed contained a greater percentage of non-extractable residue than pigweed or lambsquarter. The rapid metabolism of ethofumesate by the tolerant sugarbeet and ragweed, particularly in the leaf tissue, appeared related to tolerance. Seedlings of the highly susceptible pigweed and lambsquarter species absorbed greater amounts of $^{14}$C-ethofumesate from foliar application than the moderately susceptible ragweed and tolerant sugarbeet. Very little $^{14}$C was translocated from treated foliage to untreated plant tissue of sugarbeet. All weed species translocated $^{14}$C-ethofumesate to untreated leaf tissue when $^{14}$C-ethofumesate was applied to seedlings at the two-leaf stage. Only two-leaf pigweed and lambsquarter seedlings moved ethofumesate basipetally to the stem and root components. High percentages of $^{14}$C complexed with polar plant constituents in sugarbeet seedlings. $CO_2$ uptake and evolution were inhibited for both pigweed and sugarbeet leaves but recovered rapidly in sugarbeet, depending on age of plant at treatment. The stage of plant

development was the key factor in determining species response to foliar treatments of ethofumesate in terms of absorption, metabolism, and total photosynthesis and respiration.

To my beautiful and loving wife, Rose,

for her undaunted support of this project

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# INTRODUCTION

Sugarbeet production in Michigan is rapidly evolving toward total mechanization. The use of herbicides for weed control is often credited with providing the impetus for this change. It is estimated that 50% of the sugarbeet acreage will receive postemergence herbicides in 1978, an increase of 45% over a 10-year period, with nearly 100% receiving preemergence treatment (29). This rapid increase in the use of both pre- and postemergence herbicides is indicative of the development of successful, selective herbicides for broad spectrum weed control, and a greater understanding of the various factors influencing their selectivity.

Ethofumesate has recently been introduced for selective weed control in sugarbeet as both a soil- and foliar-applied herbicide (14,25). To help maximize weed control and minimize crop damage with ethofumesate, research was conducted to (1) determine the influence of preemergence soil-applied herbicides and timing of application on phytotoxicity of foliar ethofumesate applications in the field (2) determine the basis for increased activity with ethofumesate combinations, and (3) evaluate the physiological bases for sugarbeet tolerance to both pre- and postemergence applications of ethofumesate.

CHAPTER 1

# INFLUENCE OF PREEMERGENCE APPLIED HERBICIDES AND TIMING OF APPLICATION ON PHYTOTOXICITY OF POSTEMERGENCE HERBICIDE APPLICATIONS TO SUGARBEET (BETA VULGARIS)

## ABSTRACT

The influence of previously applied preemergence herbicides, stage of sugarbeet (Beta vulgaris L.) growth, and timing of application on activity of postemergence applications of ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate) and other herbicides used for weed control in sugarbeets were examined in the field. Preemergence application of the combination of ethofumesate plus TCA (trichloroacetic acid) caused greater growth suppression than pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) plus TCA when followed by a postemergence herbicide treatment to field grown sugarbeets. Sugarbeet tolerance was least when treatments were applied at the early two-leaf stage. Combination treatments applied with endothall [7-oxabicyclo (2,2,1) heptane-2,3-dicarboxylic acid] caused less injury. Foliar treatment at the two to four-leaf stage gave greater weed control. Mid-afternoon applications were less phytotoxic and provided equal or greater weed control compared to morning or evening treatments.

# INTRODUCTION

Total mechanization of sugarbeet production in Michigan must employ both preemergence and postemergence chemical application for weed control. Less than 10 years ago only 5% of the Michigan sugarbeets were treated with postemergence herbicides. The prediction for 1978 is that 50% will receive postemergence herbicides with nearly 100% receiving preemergence herbicides. This rapid increase in the use of both pre- and postemergence herbicides is indicative of the development of successful selective herbicides for broad spectrum weed control.

An understanding of the various factors influencing the selectivity of herbicides provides a basis for effective weed control with minimal crop damage. Ethofumesate (Figure 1) has recently been introduced for selective weed control in sugarbeets (7,12). Preemergence applications of ethofumesate offer weed control similar to that with pyrazon but with significantly greater persistence in the soil (14). Postemergence applications of ethofumesate in combination with desmedipham (ethyl m-hydroxycarbanilate carbanilate) control a broad spectrum of broadleaved weeds; however, this mixture at a normal use rate may injure sugarbeet seedlings (5,8,11). Tolerance of the sugarbeet to mixtures of ethofumesate and desmedipham is dependent on the stage of sugarbeet growth at the time of application and the amount of desmedipham in the mixture (8). Foliar growth is suppressed most when the herbicides are applied at the cotyledonary stage. The interaction between preplant incorporated applications of cycloate (S-ethyl N-ethylthiocyclohexanecarbamate) and postemergence applications of phenmedipham (methyl m-hydroxycarbanilate m-methylcarbanilate) demonstrated the preconditioning of sugarbeet and surviving weeds

to varying degrees of injury, depending on stage of growth (4). Environmental conditions can also alter the activity of herbicides (10). High temperature and light intensity have been shown to increase the injury to sugarbeets following treatment with phenmedipham (3) and desmedipham (2). Injury to sugarbeets treated with phenmedipham and desmedipham was greater when treatments were made in the early morning than in late afternoon (13,15). Weed control was also altered by timing of application (4,9).

The objectives of this study were to examine the influence of previously applied herbicides, stage of growth, and timing of application on phytotoxicity of postemergence herbicides used for weed control in sugarbeet.

## MATERIALS AND METHODS

Studies were conducted at various locations (Saginaw Co.-I, Lenawee Co.-II, and Tuscola Co.-III) in Michigan utilizing grower sugarbeet fields and at the Michigan State experiment station. Plot size was four or six 70-cm wide rows by 12 m long arranged in a randomized split block design with three or four replications. Sugarbeet 'USH20' seed was planted to final stand and herbicides applied broadcast with a tractor mounted sprayer delivering 215 l/ha at 2.1 $kg/cm^2$ pressure.

The interactions of preemergence herbicide treatment, stage of crop development, and postemergence herbicide treatment were evaluated in 1976 and 1977 at three locations per year. Soil texture in the plots was classified as a sandy clay loam for two locations, sandy loam for the third, and varied in organic matter content from 2.4 to 12.0%. Two

preemergence herbicide combinations, pyrazon plus TCA and ethofumesate plus TCA, were evaluated at rates dependent on organic matter content. Rates of pyrazon were 3.36, 4.48, or 6.72 kg/ha and 2.24 or 3.36 kg/ha for ethofumesate. The rate for TCA remained constant at 6.72 kg/ha. Sugarbeet seedlings were sprayed at the cotyledon to early two-leaf, two to four-leaf, and six to eight-leaf stages of growth. Weed species were approximately equal to or slightly larger in size than the sugarbeet. Five herbicides were applied in 12 combinations at rates previously shown to minimize crop injury and maximize weed control. Growth suppression, stand count, and weed control were assessed 7 to 10 days after each postemergence herbicide treatment. Handweeding and/or mechanical cultivation was employed for additional weed control after completion of evaluations to eliminate the effect of competition on yield. Sugarbeet roots were harvested and weighed in late October. Juice (120 ml) from samples of roots was taken from plots at two locations and analyzed for percent recoverable sugar at Michigan Sugar Company, Saginaw, Michigan. All data except for yields are expressed as percent of the non-treated control and averaged over two years and three locations.

The study on the interaction of time-of-day at postemergence treatment and herbicide was evaluated at one location in 1977. Pyrazon plus TCA (2.2 + 6.72 kg/ha) was applied preemergence to a sandy loam soil with 1.5% organic matter. Two to four-leaf sugarbeets and 5 to 8 cm tall weeds were sprayed mid-morning (9-10 am), mid-afternoon (2-3 pm), and evening (7-8 pm). Postemergence herbicide applications were made on June 13, a sunny, cloudless day with temperatures of 24°C at 10 am, 32°C at 3 pm, and 26°C at 8 pm. The temperature reached a high of 31°C the following day. Wind velocity was measured at 7.4 to 11.1 km/hr peaking at 11 am.

There was 0.38 cm rainfall 4 hr prior to the first application and no rainfall for 72 hr thereafter.

## RESULTS AND DISCUSSION

Sugarbeet injury from postemergence herbicide applications was associated with the preemergence herbicide used, stage of sugarbeet growth at time of treatment, and the specific foliar herbicide applied (Tables 1 and 2). A preemergence application of ethofumesate plus TCA was responsible for greater sugarbeet growth suppression following postemergence herbicide applications than was the pyrazon plus TCA combination. This effect caused by preemergence application of ethofumesate plus TCA was evident following most of the postemergence herbicide treatments at the three stages of growth, but did not manifest itself in significant stand reduction or yield loss. For example, the highly effective ethofumesate plus desmedipham plus endothall foliar treatment applied at the two-, four-, and six-leaf stages caused 10, 46, and 30 percent more injury, respectively, following the preemergence combination of ethofumesate plus TCA. The basis for this interaction appears to be inhibition of epicuticular wax deposition on sugarbeet leaves due to preemergence application of ethofumesate which resulted in enhanced uptake of foliar applied chemicals (6).

Sugarbeet injury was greatest when postemergence herbicide treatments were applied to plants in the early two-leaf stage, regardless of preemergence herbicide treatment. Similar results have been reported on sugarbeet tolerance to ethofumesate and desmedipham (8,11). Foliar growth, crop stand, and root yield were significantly reduced by seven

of the twelve postemergence herbicide treatments applied at the early two-leaf stage. Ethofumesate applied alone and in combination with oil concentrate or endothall, desmedipham plus endothall, and pyrazon plus desmedipham plus endothall provided the greatest margin of safety at the two-leaf stage. Suppression of foliar growth seldom resulted in significant stand reduction when evaluating the two to four- and six to eight-leaf stages, and never in yield reduction. This improved tolerance indicates decreased uptake or enhanced detoxication of the herbicide by the older, more mature sugarbeet foliage.

For both preemergence herbicide treatments at the three stages of sugarbeet growth, plant injury was greatest following a combination postemergence herbicide treatment. Ethofumesate plus desmedipham and desmedipham plus phenmedipham postemergence combinations were particularly injurious at the three growth stages. The exceptions were the combinations containing endothall (Tables 1 and 2). Endothall provided a protective effect, especially for treatments applied at the early two-leaf stage where potential injury was greatest. When the various growth parameters were analyzed for main treatment effects, it was found that sugarbeets were less tolerant to postemergence applications of desmedipham than ethofumesate. This difference in tolerance decreased with increased maturity of the sugarbeet.

A comparison between preemergence treatments revealed few significant differences in weed control (Tables 3 and 4). The ethofumesate plus TCA combination provided an improved margin of control for the few treatments where differences were measured, including the no postemergence treatment. A significant interaction for weed control was measured between postemergence herbicide treatment and stage of sugarbeet

development at time of application. Almost without exception, the foliar treatment at the two to four-leaf stage resulted in significantly greater weed control. The treatments most phytotoxic to the sugarbeet were also most effective for weed control regardless of preemergence treatment or stage of growth. Combination treatments of ethofumesate plus desmedipham, pyrazon plus desmedipham, and desmedipham plus phenmedipham provided improved weed control compared to singular treatments of ethofumesate or desmedipham at all growth stages. Endothall in combination with other herbicides did not diminish weed control. Therefore, endothall could effectively increase the margin of selectivity when applied to sugarbeets in Michigan.

No significant difference in recoverable sugar content was observed due to any of the main effects or treatment interactions.

Sugarbeet response and weed control were also found to be dependent on the time of day postemergence treatments were applied (Table 5). Mid-afternoon applications of the herbicide treatments resulted in less sugarbeet injury and greater weed control, except for ethofumesate applied alone. The desmedipham plus ethofumesate plus oil concentrate combination applied mid-morning resulted in significant stand loss and root yield reduction compared to the other times of application and the two herbicides alone. Apparently, yield loss without significant stand reduction was due to less weed control activity. Ethofumesate applied alone at all times of day provided greater safety but poor weed control resulting in reduced root yield.

Because most sugarbeets in Michigan are planted at a rate calculated to give the final stand, the influence of herbicide combinations and timing of foliar applications on both crop tolerance and weed control

are important. Mixtures of desmedipham plus ethofumesate or phenmedipham applied postemergence following a preemergence herbicide treatment provide broad spectrum weed control in Michigan sugarbeet fields. To maximize weed control and minimize sugarbeet injury the postemergence mixtures should be applied at the two to four-leaf stage of sugarbeet growth.

From the environmental conditions of temperature, light intensity, humidity, wind speed, and precipitation, it appears that temperature and light intensity are most related to sugarbeet injury following treatment with desmedipham and phenmedipham (1,2,15). Based on these studies, sugarbeet injury will also vary substantially on preemergence treatment and stage of growth at application.

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Table 1. Sugarbeet response to pyrazon plus TCA preemergence followed by postemergence herbicide applications at three stages of sugarbeet development.[a]

| Postemergence treatment | Rate (kg/ha) | Stage of sugarbeet growth at postemergence treatment: Growth suppression[b] (%) early two-leaf | two to four-leaf | six to eight-leaf | Crop stand[b] (%) early two-leaf | two to four-leaf | six to eight-leaf | Root yield (tons/ha) early two-leaf | two to four-leaf | six to eight-leaf |
|---|---|---|---|---|---|---|---|---|---|---|
| Desmedipham | 0.84 | 30 ij[c] | 20 fgh | 13 c-f | 72 abc | 90 f-k | 90 f-k | 42 a-d | 52 f-j | 53 g-j |
| Desmedipham + oil concentrate | 0.84+3.8L | 41 kl | 26 hi | 17 d-g | 73 a-d | 87 e-i | 97 h-k | 41 a-d | 55 ij | 50 e-j |
| Desmedipham + endothall | 0.84+0.56 | 20 fgh | 20 fgh | 7 acd | 88 e-j | 92 g-k | 93 g-k | 45 a-f | 48 d-i | 54 h-i |
| Ethofumesate | 1.68 | 13 c-f | 7 abc | 3 abc | 93 g-k | 98 ijk | 100 kj | 46 b-g | 53 g-j | 54 h-i |
| Ethofumesate + oil concentrate | 1.68+3.8L | 23 ghi | 10 bcd | 10 bcd | 90 f-k | 100 jk | 93 g-k | 48 d-i | 54 h-j | 57 j |
| Ethofumesate + endothall | 1.68+0.56 | 10 bcd | 10 bcd | 12 cde | 98 ijk | 94 g-k | 93 g-k | 45 a-f | 52 f-j | 55 ij |
| Ethofumesate + desmedipham | 1.68+0.84 | 57 n | 30 ij | 20 fgh | 61 a | 82 c-g | 95 h-k | 42 a-d | 50 e-j | 50 e-j |
| Ethofumesate + desmedipham + endothall | 1.68+0.84 +0.56 | 47 lm | 22 gh | 19 e-h | 65 ab | 87 e-i | 99 ijk | 39 ab | 54 h-j | 53 g-j |
| Pyrazon + desmedipham | 2.24+0.84 | 37 jk | 23 ghi | 7 abc | 77 b-e | 92 g-k | 98 ijk | 44 a-e | 56 j | 56 j |
| Pyrazon + desmedipham + endothall | 2.24+0.84 +0.56 | 22 gh | 18 e-h | 9 bc | 89 e-k | 93 g-k | 90 fk | 47 c-h | 55 ij | 55 ij |
| Desmedipham + phenmedipham | 0.84+0.84 | 50 mn | 40 kl | 30 ij | 65 ab | 78 c-f | 95 h-k | 38 a | 47 c-h | 48 d-i |
| Desmedipham + phenmedipham + endothall | 0.84+0.84 +0.56 | 39 k | 40 kl | 22 gh | 72 abc | 85 d-h | 101 k | 41 a-d | 48 d-i | 50 e-j |
| None | --------- | 0 a | 0 a | 0 a | 100 jk | 100 jk | 100 jk | 52 f-j | 53 g-j | 51 e-j |
| Main effect for years: | 1976 | 29 d | 19 bc | 11 a | 78 a | 89 bc | 98 c | 42 a | 52 b | 52 b |
| | 1977 | 33 d | 22 c | 15 ab | 82 ab | 92 c | 93 c | 46 a | 52 b | 54 b |
| Main effect for locations: | I | 29 e | 20 cd | 12 a | 79 a | 92 bc | 96 c | 47 ab | 53 c | 54 c |
| | II | 34 e | 22 d | 15 abc | 83 ab | 92 bc | 98 c | 43 a | 52 bc | 53 c |
| | III | 32 e | 18 bcd | 13 ab | 78 a | 90 bc | 90 bc | 44 a | 50 bc | 52 bc |

[a]Pyrazon rate varied among the locations depending on organic matter content (3.36, 4.48, or 6.72 kg/ha).

[b]Growth suppression and crop stand expressed as percent of plants not receiving preemergence herbicide treatment.

[c]Means for each parameter with the same letters within columns and rows are not significantly different using Duncan's multiple range test at the 5% level. Values are means of two years, three locations (Saginaw Co.-I, Lenawee Co.-II, and Tuscola Co.-III), and three replications.

Table 2. Sugarbeet response to ethofumesate plus TCA preemergence followed by postemergence herbicide applications at three stages of sugarbeet development.[a]

| Postemergence treatment | Rate (kg/ha) | Stage of sugarbeet growth at postemergence treatment: Growth suppression[b] (%) early two-leaf | two to four-leaf | six to eight-leaf | Crop stand[b] (%) early two-leaf | two to four-leaf | six to eight-leaf | Root yield (tons/ha) early two-leaf | two to four-leaf | six to eight-leaf |
|---|---|---|---|---|---|---|---|---|---|---|
| Desmedipham | 0.84 | 39 m-p[c] | 25 e-j | 22 e-h | 75 cde | 89 f-k | 96 i-m | 41 a-d | 54 ij | 56 j |
| Desmedipham + oil concentrate | 0.84+3.8L | 46 p-s | 34 k-n | 23 e-i | 68 bc | 86 e-j | 95 h-m | 39 abc | 47 d-i | 50 f-j |
| Desmedipham + endothall | 0.84+0.56 | 28 g-l | 32 k-m | 19 cde | 82 d-g | 90 f-l | 103 m | 44 b-f | 51 f-j | 54 ij |
| Ethofumesate | 1.68 | 22 e-h | 12 bc | 11 b | 96 i-m | 94 g-m | 99 klm | 45 c-g | 50 f-j | 53 hij |
| Ehtofumesate + oil concentrate | 1.68+3.8L | 25 e-j | 21 d-g | 9 b | 98 j-m | 98 j-m | 100 klm | 45 c-g | 51 f-j | 52 g-j |
| Ethofumesate + endothall | 1.68+0.56 | 21 d-g | 14 bcd | 10 b | 101 klm | 102 lm | 92 f-m | 47 d-i | 52 g-j | 53 hij |
| Ethofumesate + desmedipham | 1.68+0.84 | 53 st | 42 o-r | 32 klm | 68 bc | 86 e-j | 89 f-k | 37 ab | 44 b-f | 50 f-j |
| Ethofumesate + desmedipham + endothall | 1.68+0.84 +0.56 | 52 s | 41 n-r | 27 f-k | 70 bcd | 83 e-h | 91 f-m | 40 a-d | 50 f-j | 50 f-j |
| Pyrazon + desmedipham | 2.24+0.84 | 40 nq | 30 i-l | 20 def | 75 cde | 89 f-k | 93 g-m | 42 a-e | 52 g-j | 52 g-j |
| Pyrazon + desmedipham + endothall | 2.24+0.84 +0.56 | 34 k-n | 29 h-l | 21 d-g | 86 e-j | 90 f-l | 94 g-m | 44 b-f | 50 f-j | 52 g-j |
| Desmedipham + phenmedipham | 0.84+0.84 | 64 u | 47 qrs | 35 l-o | 52 a | 76 cde | 85 e-i | 36 a | 46 c-h | 50 f-j |
| Desmedipham + phenmedipham + endothall | 0.84+0.84 +0.56 | 60 tu | 48 rs | 35 l-o | 60 ab | 80 c-f | 90 f-l | 40 a-d | 49 e-j | 51 f-j |
| None | --------- | 0 a | 0 a | 0 a | 100 klm | 100 klm | 100 klm | 50 f-j | 52 g-j | 55 j |
| Main effect for years: | 1976 | 35 de | 26 bc | 18 a | 76 a | 87 bc | 92 bc | 43 a | 49 b | 52 b |
| | 1977 | 38 e | 31 cd | 22 ab | 83 ab | 93 c | 96 c | 41 a | 51 b | 54 b |
| Main effect for locations: | I | 36 d | 29 bc | 20 a | 77 a | 90 bcd | 92 bcd | 42 ab | 50 cde | 52 de |
| | II | 38 d | 27 b | 20 a | 84 ab | 93 bcd | 97 d | 39 a | 48 cd | 51 de |
| | III | 34 cd | 25 ab | 20 a | 77 a | 87 bc | 94 cd | 45 bc | 53 de | 55 e |

[a]Ethofumesate rate varied among the locations depending on organic matter content (2.4 or 3.36 kg/ha).

[b]Growth suppression and crop stand expressed as percent of plants not receiving preemergence herbicide treatment.

[c]Means for each parameter with the same letter or letters within columns and rows are not significantly different using Duncan's multiple range test at the 5% level of probability. Values are means of two years, three locations (Saginaw Co.-I, Lenawee Co.-II, and Tuscola Co.-III), and three replications.

Table 3. Weed control for pyrazon plus TCA preemergence followed by postemergence herbicide applications at three stages of sugarbeet development averaged over three locations for 2 years.

| Postemergence treatment | Rate (kg/ha) | Weed control (% of untreated control)[a] Stage of sugarbeet development at treatment[b] early two-leaf | two to four-leaf | six to eight-leaf |
|---|---|---|---|---|
| Desmedipham | 0.84 | 74 f-i | 86 k-n | 77 g-k |
| Desmedipham + oil concentrate | 0.84+3.8L | 70 e-g | 90 m-p | 80 h-l |
| Desmedipham + endothall | 0.84+0.56 | 75 f-i | 87 l-o | 73 e-i |
| Ethofumesate | 1.68 | 51 b | 67 def | 49 b |
| Ethofumesate + oil concentrate | 1.68+3.8L | 60 cd | 79 g-l | 68 def |
| Ethofumesate + endothall | 1.68+0.56 | 57 bc | 79 g-l | 64 cde |
| Ethofumesate + desmedipham | 1.68+0.84 | 89 m-p | 97 p | 89 m-p |
| Ethofumesate + desmedipham + endothall | 1.68+0.84 +0.56 | 81 i-m | 96 op | 82 i-m |
| Pyrazon + desmedipham | 2.24+0.84 | 79 g-l | 90 m-p | 72 e-h |
| Pyrazon + desmedipham + endothall | 2.24+0.84 +0.56 | 79 g-l | 97 p | 81 i-m |
| Desmedipham + phenmedipham | 0.84+0.84 | 79 g-l | 94 nop | 79 g-l |
| Desmedipham + phenmedipham + endothall | 0.84+0.84 +0.56 | 76 f-j | 95 op | 84 j-m |
| None | --------- | 38 a | 34 a | 38 a |
| Main effect for years: | 1976 | 71 a | 82 b | 73 a |
| | 1977 | 70 a | 85 b | 72 a |
| Main effects for locations: | I | 67 a | 81 bc | 69 a |
| | II | 71 a | 83 c | 75 ab |
| | III | 72 a | 86 c | 74 ab |

[a]Weed species evaluated include predominantly redroot pigweed, (Amaranthus retroflexus L.), common lambsquarter (Chenopodium album L.), and barnyardgrass (Echinochloa crusgalli L.) with some Pennsylvania smartweed (Polygonum pennsylvanicum L.).

[b]Means with the same letters are not significantly different at the 5% level of probability using Duncan's multiple range test.

Table 4. Weed control for ethofumesate plus TCA preemergence followed by herbicide applications at three stages of sugarbeet development averaged over three locations for 2 years.

| Postemergence treatment | Rate (kg/ha) | Weed control (% of untreated control)[a] Stage of sugarbeet development at treatment[b] early two-leaf | two to four-leaf | six to eight-leaf |
|---|---|---|---|---|
| Desmedipham | 0.84 | 72 def | 93 nop | 74 d-g |
| Desmedipham + oil concentrate | 0.84+3.8L | 84 i-m | 87 j-n | 83 h-l |
| Desmedipham + endothall | 0.84+0.56 | 72 def | 92 m-p | 78 e-j |
| Ethofumesate | 1.68 | 61 bc | 73 d-g | 65 bcd |
| Ethofumesate + oil concentrate | 1.68+3.8L | 61 bc | 74 e-h | 71 cde |
| Ethofumesate + endothall | 1.68+0.56 | 61 bc | 84 i-n | 65 bcd |
| Ethofumesate + desmedipham | 1.68+0.84 | 80 f-k | 96 op | 90 l-o |
| Ethofumesate + desmedipham + endothall | 1.68+0.84 +0.56 | 88 k-o | 97 op | 83 i-m |
| Pyrazon + desmedipham | 2.24+0.84 | 82 g-l | 92 m-p | 84 i-m |
| Pyrazon + desmedipham + endothall | 2.24+0.84 +0.56 | 81 g-l | 96 op | 85 i-n |
| Desmedipham + phenmedipham | 0.84+0.84 | 78 e-i | 97 op | 89 k-o |
| Desmedipham + phenmedipham + endothall | 0.84+0.84 +0.56 | 85 i-n | 99 p | 90 l-o |
| None | --------- | 46 a | 50 a | 47 a |
| Main effects for years: | 1976 | 72 a | 88 b | 79 a |
| | 1977 | 74 a | 87 b | 76 a |
| Main effect for locations: | I | 70 a | 85 cd | 75 ab |
| | II | 74 ab | 88 d | 80 bc |
| | III | 74 ab | 89 d | 78 bc |

[a]Weed species evaluated include predominantly redroot pigweed (Amaranthus retroflexus L.), common lambsquarter (Chenopodium album L.), and barnyardgrass (Echinochloa crusgalli L.) with some Pennsylvania smartweed (Polygonum pennsylvanicum L.).

[b]Means with the same letters are not significantly different at the 5% level of probability using Duncan's multiple range test.

Table 5. Sugarbeet response and weed control for postemergence herbicide applications at three times of day in East Lansing, Mich., 1977.

| Time of day | Postemergence[a] treatment | Rate (kg/ha) | % of untreated control[b] Growth suppression | Crop stand | Crop yield | Weed[c] control |
|---|---|---|---|---|---|---|
| Morning | Ethofumesate | 1.68 | 8 ab | 103 c | 80 b | 64 b |
| | Desmedipham | 0.84 | 40 d | 81 ab | 85 bc | 65 b |
| | Ethofumesate + Desmedipham | 1.68+0.84 | 65 e | 69 a | 65 a | 95 e |
| | None | --------- | 0 a | 100 c | 100 ef | 22 a |
| Afternoon | Ethofumesate | 1.68 | 8 ab | 98 bc | 91 cde | 74 c |
| | Desmedipham | 0.84 | 13 bc | 102 c | 89 bcd | 79 cd |
| | Ethofumesate + Desmedipham | 1.68+0.84 | 38 d | 85 b | 96 def | 95 e |
| | None | --------- | 0 a | 99 bc | 101 f | 27 a |
| Evening | Ethofumesate | 1.68 | 8 ab | 96 bc | 87 bcd | 63 b |
| | Desmedipham | 0.84 | 20 c | 96 bc | 89 bcd | 67 b |
| | Ethofumesate + Desmedipham | 1.68+0.84 | 33 d | 103 c | 91 cde | 85 d |
| | None | --------- | 0 a | 100 c | 102 f | 21 a |

[a]Postemergence treatments were applied in combination with 3.8L oil concentrate at the two to four-leaf stage. All plots were treated with pyrazon plus TCA (2.24+6.72 kg/ha) preemergence applied).

[b]Values are means of four replications and those with the same letter or letters within columns are not significantly different at the 5% level using Duncan's multiple range test.

[c]Weed species were redroot pigweed (Amaranthus retroflexus L.) and common lambsquarter (Chenopodium album L.). Plots receiving no postemergence treatment were handweeded after measurements were completed.

# ETHOFUMESATE

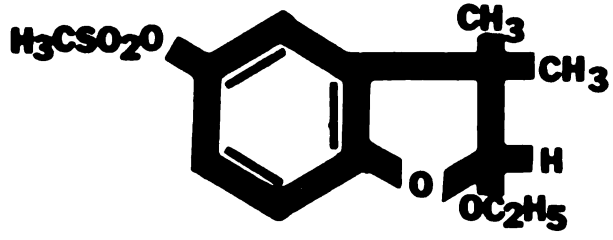


**Figure 1. Molecular structure of ethofumesate**

CHAPTER 2

# BASIS FOR INCREASED ACTIVITY FROM HERBICIDE COMBINATIONS WITH ETHOFUMESATE ON SUGARBEET (BETA VULGARIS)

## ABSTRACT

Differences in susceptibility of sugarbeet (Beta vulgaris L.) to preemergence application of ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate), pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridozinone) and TCA (trichloroacetic acid) were evaluated in several combination treatments. Exposure of plants to ethofumesate severely decreased the epicuticular wax deposition on the leaf surfaces. Separation of epicuticular wax into major components by gas-liquid chromatography indicated that ethofumesate decreased the deposition of the alkanes and sec-ketones but increased that of long-chain waxy esters. TCA also decreased the deposition of the alkane and ketone components but not of the waxy esters. Waxes were unaffected by pyrazon. Scanning electron micrographs and cuticular transpiration data further substantiated the inhibition of wax by ethofumesate. Differential inhibition of wax showed the importance of epicuticular waxes in retarding the absorption of radiolabeled herbicides. Greater absorption of $^{14}$C-ethofumesate, $^{14}$C-desmedipham, and $^{14}$C-ethofumesate + $^{14}$C-desmedipham was observed in plants that received preemergence treatments of ethofumesate and TCA as compared to pyrazon or a control. The basis for the observed interaction of

sequential herbicide combinations was increased absorption of foliar applications due to reduction in epicuticular leaf wax deposition from soil-applied herbicides.

## INTRODUCTION

Current weed control practices in sugarbeet frequently result in sequential herbicide combinations. For example, postemergence herbicides may follow the use of preplant incorporated or preemergence herbicides. Whenever herbicide combinations are used, the potential for interaction exists. The ramifications of these interactions have recently been reviewed by Putnam and Penner (10).

It has been reported that soil treatments of ethofumesate reduced the weight of epicuticular wax on the surface of cabbage (Brassica oleracea var. capitata) leaves (8). In addition, preliminary field investigations indicated ethofumesate-treated sugarbeets were less tolerant to foliar applied herbicides than were pyrazon-treated plants. This study was designed to determine the basis for this difference in susceptibility.

## MATERIALS AND METHODS

### Field Studies

Trials were conducted at two locations (Bay Co.-I and Lenawee Co.-II) in Michigan in 1976 and 1977 to evaluate combination effects of preemergence and postemergence herbicide applications on sugarbeet. Two preemergence combinations, pyrazon plus TCA and ethofumesate plus TCA, were

evaluated. Rates of pyrazon were either 3.36 or 4.48 kg/ha and for ethofumesate, 2.24 or 3.36 kg/ha depending on soil type and organic matter content. TCA was applied at 6.72 kg/ha. The postemergence treatment evaluated was the combination ethofumesate plus desmedipham at 1.68 + 0.84 kg/ha at the two to four-leaf stage. Plot size was four or six 70-cm wide rows and 12 m in length arranged in a randomized complete block design with three replications. Sugarbeet 'USH 20' seed was planted to final stand, and herbicides applied broadcast with a tractor-mounted sprayer delivering 215 l/ha at 2.1 $kg/cm^2$ pressure. Growth reduction ratings were taken 10 to 14 days after postemergence herbicide treatment. Root yield was evaluated in late October. Handweeding and/or mechanical cultivation was employed to eliminate the effect of weed competition on yield.

## Greenhouse and Laboratory Studies

Sugarbeet 'USH 20' seed were planted in 946-ml waxed cups filled with greenhouse potting soil (8% organic matter). Herbicides and herbicide mixtures were sprayed preemergence on the soil surface in an experimental spray chamber at a volume of 950 l/ha and a pressure of 2.1 $kg/cm^2$ with an 80-degree nozzle. Plants were grown outdoors or in the greenhouse supplemented with mercury halide lamps to provide a 16 hr day and increase epicuticular wax deposition on leaf surfaces. The emulsifiable formulation of ethofumesate and liquid concentrate formulation of sodium trichloroacetate (TCA) were used with an active ingredient content of 21 and 47% (w/v), respectively. A 75.5% wettable powder formulation of pyrazon was used. Experiments were conducted in a randomized complete block design

with four replications and repeated twice. All data was analyzed by computer for variance and separation of means.

Analysis of epicuticular waxes. When the sugarbeet plants reached the fully-expanded two-leaf to early four-leaf stage of development, the first two leaves were excised and allowed to wilt slightly to insure stomatal closure. Leaves were dipped for 5 sec in each of three successive 100 ml portions of glass distilled chloroform and the extract combined. Leaf area was measured with an automatic area meter (Lambda Instruments). Values reported are the means of two experiments, four replications per treatment with 32 leaves per replication.

A 30 ml aliquot of the chloroform extract of the epicuticular wax was filtered through Whatman No. 1 filter paper, evaporated at room temperature under a forced air stream, and the wax redissolved in one ml of chloroform for gas-liquid chromatography (GLC) analysis. The GLC system used a hydrogen flame detector interfaced with a recorder and Digital PDP II computer. The column was 1.7 m in length and packed with 3% Dexil 300. The column temperature was 260°C, detector temperature 360°C and inlet line 260°C. The carrier gas was nitrogen. The areas of the major peaks were measured by the computer, converted to a per $cm^2$ leaf area basis, and expressed as percent of control. The major peaks were classified by methods previously described (8). Separation of the wax extract into major chemical classes was achieved by thin layer chromatography using 250 μm silica gel G plates prewashed in benzene. The separation was visualized by spraying a 2.5 cm strip with 34 N $H_2SO_4$ and heating to 160°C for 15 min. Strips not subjected to visualization adjacent to visualized spots were scraped, eluted with 5 ml chloroform, dried, and redissolved in 1 ml chloroform for GLC analysis.

Scanning electron microscopy (SEM). Scanning electron micrographs were made with an International Scientific Instruments Super Mini-I SEM of the adaxial surface of fresh sugarbeet leaves. Micrographs were made of the second true leaf of control plants and plants receiving 2.24 kg/ha ethofumesate. Pieces (4 by 8 mm) were cut from each leaf at one side of the midvein. The leaf pieces were placed abaxial surface down upon the adhesive side of a 1 $cm^2$ strip of silver metallic tape that had been previously glued to the top of an aluminum SEM stub. The stubs were then placed in the SEM and micrographs taken on Polaroid type 107 film at 5 kv acceleration potential. The process consumed less than 10 min from the time the leaf pieces were first cut. Of primary interest was the change in fine-structure of the wax bloom of treated plants.

Cuticular transpiration. The effect of the preemergence herbicide treatment on the rate of transpiration through the cuticle was determined by measuring the rate of water loss from excised sugarbeet leaves. The first two true leaves were removed and placed in aluminum pans so as not to overlap. A dewaxed control was prepared by immersing leaves from control plants into chloroform for 15 sec. The pans were weighed at 0 and 1, 2, 4, 8, and 24 hr after exposure to these conditions. The weight of water lost from the leaves during each time period was calculated. A regression coefficient (equal to percent water lost per hr) was calculated for each treatment from initiation of the experiment until 90% of the original water was lost or 24 hr, whichever came first. Stomata on the excised leaves closed after 0.3 hr. Values presented are the mean of four replications.

Foliar uptake of $^{14}C$-ethofumesate and $^{14}C$-desmedipham. Ethofumesate was uniformly labeled in the benzofuranyl ring (sp. act. 1.34 μCi/mole).

Desmedipham was radiolabeled with $^{14}C$ in the m-aminophenol ring (sp. act. 1.95 μCi/mole). The radiolabeled herbicides were dissolved in ethanol and additional formulated herbicide added to obtain the desired concentrations. A 10 ul drop containing 0.1 μCi of herbicide was placed on the upper leaf surface of the first two true leaves and spread uniformly with the rounded end of a glass rod. Concentrations approximated the use rate for foliar application. At the two sampling dates, 3 and 24 hr, the treated leaves of each plant were harvested and rinsed with 20 ml of 50% ethanol to remove any remaining herbicide. After the leaf area was measured, the leaves were frozen on dry ice and then freeze-dried. A Packard 306 Tri-Carb Sample Oxidizer was used to combust the tissue. Released $^{14}CO_2$ was trapped with 12 ml Carbo-sorb$^R$ and further diluted with 10 ml Permafluor$^R$ V liquid scintillation cocktail for carbon-14. The vials containing the sample were radioassayed for 10 min with a liquid scintillation spectrometer. Data is expressed as percent $^{14}C$ absorbed of the total applied to the leaf.

## RESULTS

### Field Studies

Reduction of sugarbeet growth was significantly greater when the postemergence combination of ethofumesate plus desmedipham was applied subsequent to a preemergence herbicide treatment compared to the preemergence only treatment (Table 1). Since the postemergence herbicide treatment caused no apparent visual difference among plants not receiving a preemergence treatment, this increased injury (1 to 18% for pyrazon + TCA and 3 to 32% for ethofumesate + TCA), can be attributed to a preconditioning

of the sugarbeet by the preemergence herbicide. Root yields were unaffected by the treatments. Visual observations revealed an ethofumesate-induced leaf surface alteration on sugarbeet (Figure 1). Leaves appeared glossy when compared to those receiving a preemergence treatment of pyrazon.

## Greenhouse and Laboratory Studies

Wax studies. Sugarbeet leaf wax was separated into three major components and identified by GLC procedures (Table 2). The ethofumesate treatment significantly decreased the deposition of C-29 alkane and sec-tone components as compared to the control and pyrazon treatments. TCA, a known inhibitor of leaf waxes (1,6,7), also reduced these long-chain wax fractions. In combination with ethofumesate, TCA had little effect on the epicuticular wax content when compared with ethofumesate alone; but in combination with pyrazon, TCA significantly altered the wax deposition compared to pyrazon alone. Ethofumesate applied alone and in combination with TCA significantly increased the deposition of long-chain waxy esters, a component comprising only 5% of the total recovered by GLC.

Scanning electron micrographs of sugarbeet epicuticular wax from ethofumesate treated and non-treated leaves are shown in Figure 2. The crystalline fine-structure was entirely eliminated by preemergence ethofumesate treatments.

Cuticular transpiration of sugarbeet leaves was increased significantly by all treatments except pyrazon (Table 3). With exception of the chloroform-washed control, the two ethofumesate treatments lost the greatest percentage of water per hour (15.9% for ethofumesate and27.9%

for ethofumesate plus TCA).

Postemergence herbicide absorption study. Contrary to a previous report (3), single applications of $^{14}$C-ethofumesate and $^{14}$C-desmedipham were similarly absorbed, both for the control and preemergence herbicide treated plants (Tables 3 and 4). The exception was the increased absorption of ethofumesate when applied over ethofumesate preemergence-treated sugarbeets. For all preemergence herbicide treatments the combination postemergence treatment resulted in significantly greater absorption compared to application of individual postemergence herbicides. Ethofumesate pre-treatments caused the greatest increase in foliar absorption of the $^{14}$C-herbicides. Only the preemergence application of the ethofumesate plus TCA combination caused significantly greater $^{14}$C absorption than that obtained from each component of the mixture. The TCA in the pyrazon plus TCA combination contributed most to the increased absorption of all foliar treatments. Other than a general increase in uptake after 24 hr of exposure, no major differences were noted between the two times of exposure (Tables 4 and 5).

## DISCUSSION

Data from the 2-year field study confirmed the ethofumesate-induced preconditioning of sugarbeet to increased growth reduction upon foliar treatment. Based on the glossy appearance of ethofumesate treated leaves, the hypothesis that such treatments reduced epicuticular wax production to cause increased susceptibility to herbicide sprays was tested.

Data from the various aspects of the wax study clearly indicate marked inhibition by ethofumesate of deposition of epicuticular waxes on

developing sugarbeet leaves. Although ethofumesate differs considerably from EPTC (S-ethyl-N,N-di-n-propyl thiocarbamate) and TCA in structure, it had a similar and even greater effect on epicuticular wax deposition. Because ethofumesate increased the deposition of long-chain esters and decreased the C-29 alkane and C-29 sec-ketone components, the mechanism of action could be explained by inhibition of fatty acid elongation in the elongation-decarboxylation pathway of epicuticular wax synthesis. Similar interpretations have been advanced by Leavitt et al. (8) for ethofumesate-induced alterations in wax and by Flore (4) for EPTC-induced surface wax alterations on cabbage. In addition to a reduction in epicuticular wax production, ethofumesate caused changes in the surface fine-structure (Figure 2).

The preemergence herbicide treatments reduced deposition of major wax components and increased cuticular evapotranspiration. The decrease in epicuticular wax caused greater absorption of foliar applied $^{14}C$-herbicide, both alone and as combinations, when applied to the first two true leaves of plants in the early four-leaf stage of development. This would explain the herbicide interaction observed in the field study. Similarly, increased sensitivity of EPTC and TCA treated plants to subsequent herbicide sprays (2,5,9) suggested increased penetration. Davis and Dusbabek (1) demonstrated increased uptake of $^{14}C$-pesticides by peas (Pisum sativum L.) exposed to the thiocarbamate, diallate [S-(2,3-dichloroallyl)diisopropylthiocarbamate].

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Table 1. Influence of preemergence herbicide treatment on the activity of ethofumesate plus desmedipham combination applied postemergence in field studies at 1.68 + 0.84 kg/ha during 1976 and 1977.

| Treatment | | Growth reduction (%) | Root yield (ton/ha) |
|---|---|---|---|
| Preemergence[a] | Postemergence[b] | | |
| - | - | 0 a[c] | 54 a |
| - | + | 4 a | 54 a |
| Pyrazon + TCA | - | 1 a | 52 a |
| Pyrazon + TCA | + | 18 b | 50 a |
| Ethofumesate + TCA | - | 3 a | 52 a |
| Ethofumesate + TCA | + | 32 c | 47 a |
| Main effect for years: | 1976 | 6.4 a | 54 a |
| | 1977 | 13.1 a | 49 a |
| Main effect for locations: | I | 8.9 a | 52 a |
| | II | 11.0 a | 50 a |

[a]Preemergence herbicide rate varied between two locations depending on soil type and organic content.

[b]Postemergence treatment of ethofumesate plus desmedipham applied to sugarbeets in the two to four-leaf stage.

[c]Values within columns with the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 2. Effect of preemergence herbicide treatment on major sugarbeet wax components determined by GLC.

| Treatment | Rate (kg/ha) | % of control[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | C-29 alkane | | C-29 sec-ketone | | long-chain waxy esters | |
| Ethofumesate | 2.24 | 9 | a | 12 | a | 150 | b |
| Pyrazon | 3.36 | 94 | c | 85 | c | 103 | a |
| TCA | 6.72 | 41 | b | 55 | b | 89 | a |
| Ethofumesate + TCA | 2.24+6.72 | 3 | b | 9 | a | 135 | b |
| Pyrazon + TCA | 3.36+6.72 | 49 | b | 61 | b | 95 | a |

[a]Values within columns with the same letter are not significantly different at the 5% level using Duncan's multiple range test. For the control, the C-29 alkane comprised 61%, C-29 sec-ketone comprised 20%, and long-chain waxy esters comprised 5% of that recovered by the GLC system.

Table 3. Cuticular transpiration of sugarbeet leaves in response to preemergence herbicide treatment.

| Treatment | Rate (kg/ha) | Transpiration (% $H_2O$ loss/hr)[a] |
|---|---|---|
| Control | - | 4.3 a |
| $CHCl_3$-washed control | - | 51.5 e |
| Ethofumesate | 2.24 | 15.9 c |
| Pyrazon | 3.36 | 3.8 a |
| TCA | 6.72 | 10.0 b |
| Ethofumesate + TCA | 2.24+6.72 | 27.9 d |
| Pyrazon + TCA | 3.36+6.72 | 12.1 bc |

[a]Values with the same letter or letters are not significantly different at the 5% level using Duncan's multiple range test.

Table 4. Influence of preemergence treatment on absorption of $^{14}$C-herbicides after 3 hr exposure.

| Preemergence Treatment | Rate (kg/ha) | % $^{14}$C absorbed of total applied[a] Foliar treatment (kg/ha) $^{14}$C-Ethofumesate (1.69) | $^{14}$C-Desmedipham (0.84) | $^{14}$C-Eth+$^{14}$C-Des (1.68+0.84) |
|---|---|---|---|---|
| Control | - | 23 a | 31 b | 50 ef |
| Ethofumesate | 2.24 | 50 ef | 42 cd | 62 g |
| Pyrazon | 3.36 | 30 b | 35 bc | 51 ef |
| TCA | 6.72 | 42 cd | 42 cd | 56 fg |
| Ethofumesate + TCA | 2.24+6.72 | 60 g | 52 ef | 76 h |
| Pyrazon + TCA | 3.36+6.72 | 46 de | 48 de | 50 g |

[a]Values with the same letter or letters within rows and columns are not significantly different at the 5% level using Duncan's multiple range test.

Table 5. Influence of preemergence treatment on absorption of $^{14}$C-herbicides after 24 hr exposure.

| Preemergence Treatment | Rate (kg/ha) | % $^{14}$C absorbed of total applied[a] Foliar treatment (kg/ha) $^{14}$C-Ethofumesate (1.69) | $^{14}$C-Desmedipham (0.84) | $^{14}$C-Eth+$^{14}$C-Des (1.68+0.84) |
|---|---|---|---|---|
| Control | - | 62 b | 58 a | 75 de |
| Ethofumesate | 2.24 | 82 fg | 78 de | 90 g |
| Pyrazon | 3.36 | 72 cd | 64 b | 79 ef |
| TCA | 6.72 | 76 de | 72 cd | 83 fg |
| Ethofumesate + TCA | 2.24+6.72 | 91 g | 80 ef | 96 h |
| Pyrazon + TCA | 3.36+6.72 | 77 de | 70 cd | 89 g |

[a]Values with the same letter or letters within rows and columns are not significantly different at the 5% level using Duncan's multiple range test.

Figure 1. Photograph of upper surface of first two true sugarbeet leaves. Control (1) and ethofumesate-treated preemergence at 2.24 kg/ha (r).

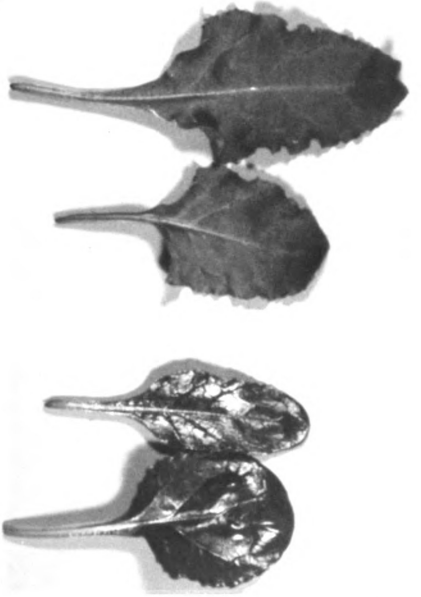
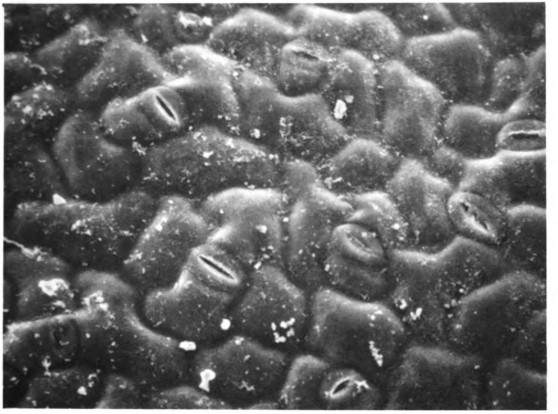
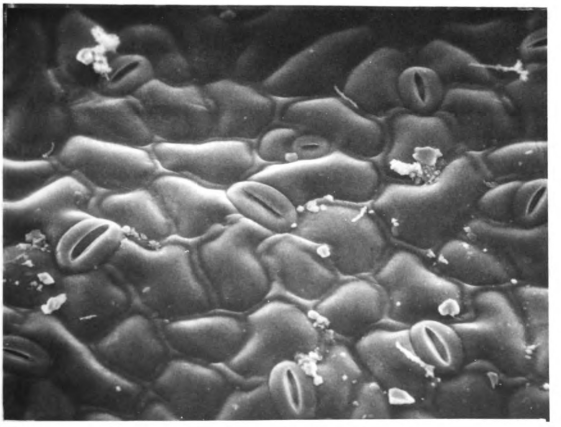


Figure 2. Scanning electron micrographs of the upper surface of fresh sugarbeet leaves from control (above) and ethofumesate-treated (below) plants, 1000x.





CHAPTER 3

# THE BASIS FOR SELECTIVITY OF ROOT-APPLIED ETHOFUMESATE IN SUGARBEET AND THREE WEED SPECIES

## ABSTRACT

The absorption, translocation, and metabolism of $^{14}$C-ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate) in sugarbeet (Beta vulgaris L.), common ragweed (Ambrosia artemisfolia L.), redroot pigweed (Amaranthus retroflexus L.), and common lambsquarter (Chenopodium album L.) were studied as possible bases for selectivity of preemergence applied ethofumesate. The seedlings were grown in the greenhouse in nutrient culture and transferred to nutrient solution containing $^{14}$C-ethofumesate for exposures of 1, 3, or 7 days. The sensitive pigweed and lambsquarter plants translocated more $^{14}$C-ethofumesate to the leaf tissue than the tolerant sugarbeet and ragweed. The $^{14}$C was more highly concentrated in the root portion of sugarbeet and ragweed. Movement and accumulation increased with time of exposure for all species. Stem tissue of both sugarbeet and ragweed contained a greater percentage of non-extractable residue than pigweed or lambsquarter. There were considerable species differences in the $^{14}$C-metabolites formed after 1, 3, and 7 days exposure to $^{14}$C-ethofumesate. The rapid metabolism of ethofumesate by the tolerant sugarbeet and ragweed, particularly in the leaf tissue, appeared related to tolerance.

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## INTRODUCTION

Ethofumesate is a new selective herbicide recently registered[1] by the Environmental Protection Agency for preplant incorporation and preemergence control of some annual broadleaf and grass weeds in sugarbeet (5,6). Redroot pigweed and common lambsquarter are highly susceptible to preemergence applications of ethofumesate. However, common ragweed is equal to the sugarbeet in tolerance (2,3). No information is available on the mechanism for this difference in tolerance.

The purpose of this study was to determine the basis for the selectivity of ethofumesate between sugarbeet, redroot pigweed, common lambsquarter, and common ragweed by comparing root absorption, translocation, and metabolism.

## MATERIALS AND METHODS

'USH 20' sugarbeet, common ragweed, redroot pigweed, and common lambsquarter were germinated in vermiculite in the greenhouse and transferred to 1/2-strength Hoagland's nutrient solution (4) in foil wrapped, 250-ml plastic pots with sponge supports. When plants were in the cotyledon stage they were placed in growth chambers with a 16 hr day at 25 C. Light intensity at plant level was 24 to 27 klux provided by a mixture of fluorescent and incandescent lamps. The $^{14}$C-ethofumesate was uniformly labeled in the benofuranyl ring with a specific activity of 1.34 μCi/μmole. A stock solution was made by dissolving the ethofumesate in ethanol and

[1]Registered as Nortron$^{R}$, a trademark of Fisons Limited.

diluting it with formulated herbicide in distilled water. The ethanol was evaporated with nitrogen before application. Data reported are the means of two experiments with four replications each. After a 7 day period of acclimatization and a selection for uniformity, plants were transferred to foil-lined, 50 ml graduated tubes (one per tube) containing $4 \times 10^{-6}$ M ethofumesate and nutrient solution. Transpiration was monitored over time to determine if species differences existed between water uptake and herbicide uptake. The tubes were replenished daily with the ethofumesate-containing nutrient solution. One ml aliquots were taken to chromatographically determine the % purity of the parent ethofumesate at each time interval by thin layer chromatography (TLC).

Four replicate plants were harvested after 1, 3, and 7 days exposure. One plant was used for radioautography and three for quantitative $^{14}C$ determination. At harvest, the roots were rinsed in three consecutive water baths and blotted dry. The plants were placed on dry ice, freeze-dried, and then radioautographed or divided into leaf, stem, and root sections for dry weight determination and extraction. Plant parts were homogenized twice in 50 ml 80% ethanol with a Sorvall Omni-Mixer. After grinding, the plant extracts were filtered through Whatman No. 1 filter paper under vacuum and rinsed with ethanol. The ethanol-insoluble residue was collected for dry weight determination and combusted to quantitatively determine the ethanol-insoluble $^{14}C$ material. A Packard 306 Tri-Carb Sample Oxidizer was used to combust the tissue. Released $^{14}CO_2$ was trapped with 12 ml Carbo-Sorb$^R$ and further diluted with 10 ml Permaflour$^R$ V liquid scintillation solution. The ethanol-soluble filtrate was evaporated in vacuo and the remaining aqueous residue partitioned twice with 40 ml dichloromethane. The dichloromethane phase was sampled for radioactivity

and chromatographic determination of $^{14}C$-ethofumesate and $^{14}C$-metabolites. The aqueous layer was boiled for 2 hr with 12 N HCL to hydrolize any conjugates. After another extraction and partitioning with dichloromethane, aliquots were taken of both aqueous and dichloromethane layers for additional radioassay and chromatography of metabolites released from conjugation. All aliquots were radioassayed by liquid scintillation spectrometry and 100 µl of each fraction spotted on 250 µmeter think silica gel G thin layer chromatography plates. The plates were radioautographed and the $^{14}C$-labeled spots on the plate removed and radioassayed. All radioassay data was corrected for background with efficiency determined by channels ratio method.

## RESULTS AND DISCUSSION

During the first 3 days of exposure, ragweed and lambsquarter absorbed significantly more $^{14}C$-ethofumesate from the nutrient solution than sugarbeet (Table 1). Sugarbeet, an ethofumesate tolerant species, absorbed low amounts of $^{14}C$. The highly susceptible redroot pigweed absorbed a similar amount during this time. The greatest amount of $^{14}C$ was found in the highly tolerant common ragweed while the susceptible lambsquarter ranked intermediate in $^{14}C$ uptake. After 7 days there were no differences in absorption among species. Therefore, these results indicate that differences in root absorption of $^{14}C$-ethofumesate do not explain differences in ethofumesate selectivity between these two tolerant and two susceptible species.

The distribution pattern of $^{14}C$-ethofumesate indicates rapid translocation of $^{14}C$ to the leaf portion of the susceptible redroot pigweed

and lambsquarter species (Table 2). The $^{14}C$ was more highly concentrated in the root portion of the tolerant sugarbeet and common ragweed species. The movement and accumulation increased with time for all species (Table 2 and Figures 1 to 4). It has been reported that foliar treatments of ethofumesate act to inhibit photosynthesis and respiration in redroot pigweed (2). Conceivably, greater transport of $^{14}C$-ethofumesate to the leaf portion of the susceptible redroot pigweed and lambsquarter could, in part, account for response differences between susceptible and tolerant plants.

Analysis of the ethanol-insoluble (non-extractable) $^{14}C$-labeled residue revealed a significant interaction among species, plant component, and length of exposure time (Table 3). Both sugarbeet and ragweed contained a greater percentage of radioactivity in the ethanol-insoluble fraction of the stem than either the leaf or root segments. The stem portion of the tolerant species was higher in radioactivity than that of the two susceptible species. This response was particularly evident for ragweed at all times for sugarbeet after 3 days exposure. Thus, the rapid $^{14}C$ uptake by ragweed was counteracted by metabolism or binding of $^{14}C$ into the ethanol-insoluble fraction of the stem and leaf.

TLC analysis of crude ethanol/dichloromethane extracts of plants following root treatment with $^{14}C$-ethofumesate provides evidence for metabolism of ethofumesate by all species (Tables 4, 5, and 6). After 7 days exposure, ethofumesate metabolism in sugarbeet and ragweed differed markedly from that of redroot pigweed and lambsquarter. Only a small percentage of $^{14}C$ was detected in the dichloromethane-soluble fraction, particularly after 7 days exposure, in sugarbeet compared to the other three species (Table 5). The majority of the $^{14}C$ was possibly conjugated

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with water-soluble plant constituents. Acid hydrolysis of the water-soluble fraction of leaf components released decreasing amounts of parent ethofumesate with increased time of exposure for sugarbeet (85% to 11%) and ragweed (69% to 36%). The opposite was observed for leaves of pigweed (21% to 79%) and lambsquarter (40% to 75%) (Table 7). For both sugarbeet and ragweed, the metabolism of parent ethofumesate increased with time of exposure, particularly in the leaf segment (Table 6). The percentage of $^{14}C$ remaining as unmetabolized ethofumesate was significantly greater in susceptible redroot pigweed and lambsquarter than for the two tolerant species. This difference was specially evident after 7 days exposure.

Differences in phytotoxicity of root-applied ethofumesate appears to be dependent on both metabolism and translocation within the plant species. Although both sugarbeet and ragweed are resistant species, they appear to have differing mechanisms for detoxifying the parent ethofumesate. In sugarbeet there was rapid conjugation with dichloromethane-insoluble plant constituents and relatively slower absorption than in the susceptible species. And after 7 days, hydrolysis revealed only 11% parent ethofumesate remaining in the leaf portion of the dichloromethane-soluble fraction (Table 7). Conversely, ragweed rapidly converted parent ethofumesate into dichloromethane-soluble metabolites. Few water-soluble products were formed. Extremely slow metabolism in all plant parts contributed to the observed susceptibility of redroot pigweed and lambsquarter to ethofumesate.

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Table 1. Uptake of $^{14}$C-ethofumesate by sugarbeet and three weed species grown in nutrient solution containing 4 x $10^{-6}$ M ethofumesate.

| Species | Time of exposure | | |
|---|---|---|---|
| | 1 day | 3 day | 7 day |
| | (μmole/mg whole plant dry wt) | | |
| Sugarbeet | 25 a[a] | 41 ab | 83 de |
| Ragweed | 69 cd | 90 e | 88 de |
| Pigweed | 38 ab | 56 bc | 81 de |
| Lambsquarter | 49 bc | 67 cd | 71 cde |

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

Tab

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Table 2. Distribution of $^{14}C$ in sugarbeet and three weed species grown in nutrient solution containing 4 x $10^{-6}$ M ethofumesate after 1, 3, and 7 days exposure.

| Species | Plant component | Time of exposure | | |
|---|---|---|---|---|
| | | 1 day | 3 day | 7 day |
| | | (μmole/mg dry wt) | | |
| Sugarbeet | Leaf | 29 ab[a] | 47 b-e | 77 h-k |
| | Stem | 21 a | 20 a | 76 g-k |
| | Root | 21 a | 33 ab | 103 mn |
| Ragweed | Leaf | 45 bcd | 67 f-i | 69 f-j |
| | Stem | 100 lmn | 88 klm | 83 i-l |
| | Root | 129 o | 165 p | 169 p |
| Pigweed | Leaf | 87 j-m | 78 h-k | 114 no |
| | Stem | 65 e-i | 58 d-g | 83 i-f |
| | Root | 30 ab | 54 c-f | 53 c-f |
| Lambsquarter | Leaf | 56 c-f | 53 c-f | 93 klm |
| | Stem | 45 bcd | 34 ab | 67 f-i |
| | Root | 22 a | 38 abc | 61 d-h |

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

Table 3. Percent non-extractable radioactivity after $^{14}$C-ethofumesate absorption for leaf, stem, and root components of 4 species after 1, 3, and 7 days exposure.

| Species | Length of exposure (days) | Plant component | | |
|---|---|---|---|---|
| | | Leaf (%) | Stem (%) | Root (%) |
| Sugarbeet | 1 | 18 a-e[a] | 18 a-e | 6 ab |
| | 3 | 24 c-g | 41 hi | 17 a-e |
| | 7 | 38 gh | 66 k | 16 a-e |
| Ragweed | 1 | 8 ab | 35 fgh | 5 a |
| | 3 | 28 d-h | 60 jk | 8 ab |
| | 7 | 53 ij | 70 k | 14 a-d |
| Pigweed | 1 | 16 a-e | 20 b-e | 8 ab |
| | 3 | 29 e-h | 18 a-e | 16 a-e |
| | 7 | 37 gh | 18 a-e | 16 a-e |
| Lambsquarter | 1 | 14 abc | 34 fgh | 13 abc |
| | 3 | 23 c-f | 33 fgh | 16 a-e |
| | 7 | 37 gh | 32 e-h | 25 c-g |

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

Table 4. Metabolites of $^{14}C$-ethofumesate and corresponding TLC Rf values obtained from leaf segments of four species harvested after 1, 3, and 7 days exposure.[a]

| Fraction | Sugarbeet metabolites | | | | Ragweed metabolites | | | | Pigweed metabolites | | | | Lambsquarter metabolites | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rf of metabolites | 1 day (%) | 3 day (%) | 7 day (%) | Rf of metabolites | 1 day (%) | 3 day (%) | 7 day (%) | Rf of metabolites | 1 day (%) | 3 day (%) | 7 day (%) | Rf of metabolites | 1 day (%) | 3 day (%) | 7 day (%) |
| Dichloromethane-soluble | .00 | 3 | 3 | 6 | .00 | 5 | 3 | 6 | .00 | 13 | 3 | 0 | .00 | 9 | 7 | 8 |
| | .07 | 10 | 19 | 34 | .05 | 17 | 25 | 20 | .07 | 2 | 4 | 4 | .07 | 7 | 9 | 8 |
| | .29 | 0 | 5 | 10 | .13 | 0 | 4 | 2 | .29 | 0 | 0 | 0 | .29 | 0 | 3 | 2 |
| | | | | | .16 | 0 | 3 | 2 | | 0 | 0 | 0 | | 0 | 1 | 2 |
| | | | | | .27 | 9 | 12 | 38 | | 0 | 0 | 0 | | 0 | 0 | 0 |
| Ethofumesate | .35 | 87 | 73 | 50 | .33 | 69 | 53 | 32 | .35 | 85 | 93 | 96 | .35 | 84 | 80 | 80 |

[a]Values are percentages of total detectable $^{14}C$ by TLC.

Table 5. Metabolism of $^{14}$C-ethofumesate in leaf, stem, and root components of four species after 1, 3, and 7 days exposure.[ab]

| Species | Plant part | Dichloromethane-soluble fraction (%) exposure (days) 1 | | 3 | | 7 | | Water-soluble residue (%) 1 | | 3 | | 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sugarbeet | Leaf | 40 | cd | 21 | ab | 10 | ab | 60 | kl | 79 | no | 90 | no |
| | Stem | 67 | f-j | 56 | ef | 9 | a | 33 | e-i | 44 | i | 91 | o |
| | Root | 85 | k-n | 63 | fgh | 55 | def | 15 | a-d | 37 | ghi | 45 | ij |
| Ragweed | Leaf | 90 | mn | 77 | g-n | 81 | j-n | 10 | ab | 23 | a-g | 19 | a-e |
| | Stem | 92 | n | 88 | lmn | 77 | g-n | 8 | a | 12 | abc | 23 | d-g |
| | Root | 89 | lmn | 89 | lmn | 84 | k-n | 11 | ab | 11 | ab | 16 | a-d |
| Pigweed | Leaf | 21 | ab | 41 | cde | 72 | g-k | 79 | no | 59 | jk | 28 | d-h |
| | Stem | 78 | h-n | 72 | g-k | 77 | g-n | 22 | a-f | 28 | d-h | 23 | a-g |
| | Root | 78 | h-n | 74 | g-l | 65 | f-i | 22 | a-g | 26 | c-h | 35 | f-i |
| Lambsquarter | Leaf | 36 | bc | 24 | ab | 76 | g-m | 64 | lm | 76 | mn | 24 | b-h |
| | Stem | 64 | f-i | 62 | fg | 79 | i-n | 34 | f-i | 38 | hi | 21 | a-f |
| | Root | 84 | k-n | 81 | j-n | 92 | n | 16 | a-d | 19 | a-e | 8 | a |

[a]Values are expressed as percent of total extractable $^{14}$C-ethofumesate.

[b]Means within each of the two fractions having identical letters are not significantly different at the 5% level using Duncan's multiple range test.

Table 6. Metabolism of $^{14}$C-ethofumesate in dichloromethane-soluble fraction as a function of plant species and component and time of exposure.[ab]

| Species | Plant component | Origin (%) | | | Metabolites (%) | | | Parent ethofumesate (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Length of exposure (days) | | | | | | | | |
| | | 1 | 3 | 7 | 1 | 3 | 7 | 1 | 3 | 7 |
| Sugarbeet | Leaf | 3 a-e | 3 a-e | 6 a-e | 10 b-e | 24 gh | 44 k | 87 j-n | 73 d-g | 50 b |
| | Stem | 1 ab | 11 c-f | 9 a-e | 13 c-f | 4 abc | 23 gh | 86 im | 85 i-l | 68 cde |
| | Root | 6 a-e | 7 a-e | 3 a-e | 15 d-g | 20 fgh | 26 hi | 79 f-j | 73 d-g | 71 d-g |
| Ragweed | Leaf | 5 a-e | 3 a-e | 6 a-e | 26 hi | 44 k | 62 l | 69 d-e | 53 b | 32 a |
| | Stem | 4 a-e | 4 a-e | 1 ab | 10 b-e | 19 e-h | 39 jk | 86 i-m | 77 e-i | 59 bc |
| | Root | 5 a-e | 3 a-e | 0 a | 26 hi | 27 hi | 34 ij | 69 d-e | 70 def | 66 cd |
| Pigweed | Leaf | 13 ef | 3 a-e | 0 a | 2 ab | 4 abc | 4 abc | 85 i-l | 93 lmn | 96 n |
| | Stem | 20 f | 12 def | 2 a | 5 abc | 2 ab | 8 a-d | 75 d-h | 86 i-m | 90 k-n |
| | Root | 10 b-e | 10 b-e | 0 a | 10 b-e | 0 a | 5 abc | 80 g-j | 90 k-n | 95 mn |
| Lambsquarter | Leaf | 9 a-e | 7 a-e | 8 a-e | 7 a-d | 13 c-f | 12 c-f | 84 h-i | 80 g-j | 80 g-j |
| | Stem | 10 b-e | 5 a-e | 6 a-e | 4 abc | 8 a-d | 11 b-f | 86 i-m | 87 j-n | 83 h-k |
| | Root | 5 a-e | 2 abc | 2 abc | 0 a | 12 c-f | 6 a-d | 95 mn | 86 i-m | 92 k-n |

[a]Values are expressed in terms of percent of total radioactivity found.

[b]Means within each category having identical letters are not significantly different at the 5% level using Duncan's multiple range test.

Table 7. Dichloromethane-soluble metabolites released by acid hydrolysis of the water-soluble fraction.[ab]

| Species | Plant component | Origin (%) | | | Metabolites (%) | | | Parent ethofumesate (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Length of exposure (days) | | | | | | | | |
| | | 1 | 3 | 7 | 1 | 3 | 7 | 1 | 3 | 7 |
| Sugarbeet | Leaf | 6 a | 5 a | 12 a | 9 abc | 33 e-i | 77 n | 85 mn | 62 g-k | 11 a |
| | Stem | 5 a | 5 a | 7 a | 65 mn | 36 e-j | 58 lm | 30 bc | 59 f-k | 35 bcd |
| | Root | 6 a | 6 a | 9 a | 64 mn | 45 h-l | 55 klm | 30 bc | 49 d-h | 36 bcd |
| Ragweed | Leaf | 16 a | 2 a | 8 a | 15 a-d | 53 j-m | 57 lm | 69 i-m | 45 c-g | 36 bcd |
| | Stem | 11 a | 16 a | 8 a | 25 b-g | 32 d-h | 41 g-l | 64 h-k | 52 d-h | 52 d-h |
| | Root | 7 a | 13 a | 7 a | 45 h-l | 22 a-f | 31 d-h | 49 d-h | 66 h-l | 62 g-k |
| Pigweed | Leaf | 15 a | 9 a | 9 a | 64 mn | 31 d-h | 12 abc | 21 ab | 60 f-k | 79 lmn |
| | Stem | 13 a | 11 a | 14 a | 11 abc | 26 c-g | 13 abc | 76 k-n | 63 h-k | 74 j-n |
| | Root | 5 a | 11 a | 5 a | 38 e-k | 8 ab | 13 abc | 58 f-j | 82 lmn | 82 lmn |
| Lambsquarter | Leaf | 10 a | 8 a | 5 a | 50 i-m | 39 f-k | 21 a-e | 40 cde | 54 e-i | 75 j-n |
| | Stem | 10 a | 5 a | 6 a | 25 b-g | 34 e-i | 7 a | 65 h-l | 61 g-k | 87 n |
| | Root | 5 a | 2 a | 3 a | 52 j-m | 37 e-j | 38 e-k | 43 c-f | 61 g-k | 59 f-k |

[a]Means within each of the three categories having identical letters are not significantly different at the 5% level using Duncan's multiple range test.

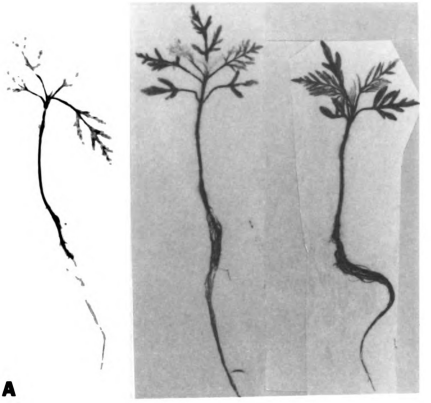
[b]Values are expressed as percent of total radioactivity found.

Figure 1. Translocation of $^{14}C$-ethofumesate in sugarbeet. The radioautograph (A) after 1, 3, and 7 days exposure to treatment (left to right) and corresponding plant specimen (B).

A

B

Figure 2. Translocation of $^{14}C$-ethofumesate in ragweed. The radio-autograph (A) after 1, 3, and 7 days exposure to treatment (left to right) and corresponding plant specimen (B).

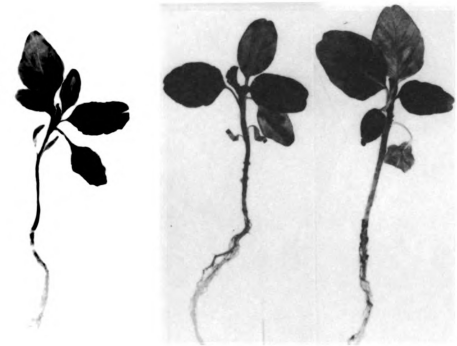


A

B

Figure 3. Translocation of $^{14}C$-ethofumesate in redroot pigweed. The radioautograph (A) after 1, 3, and 7 days exposure to treatment (left to right) and corresponding plant specimen (B).

A



B

Figure 4. The translocation of $^{14}C$-ethofumesate in lambsquarter. The radioautograph (A) after 1, 3, and 7 days exposure to treatment (left to right) and corresponding plant specimen (B).

CHAPTER 4

# PHYSIOLOGICAL BASES OF SUGARBEET (*BETA VULGARIS*) TOLERANCE TO FOLIAR APPLICATION OF ETHOFUMESATE

## ABSTRACT

Absorption, translocation, and metabolism of foliar-applied ethofumesate (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate) were studied to explain field observations showing differences in susceptibility among sugarbeet (*Beta vulgaris* L.), common ragweed (*Ambrosia artemisiifolia* L.), redroot pigweed (*Amaranthus retroflexus* L.), and common lambsquarter (*Chenopodium album* L.). In laboratory studies seedlings of the highly susceptible species, pigweed and lambsquarter, absorbed greater amounts of $^{14}C$-ethofumesate from foliar application than the moderately susceptible ragweed and tolerant sugarbeet. Sugarbeet translocated very little $^{14}C$ from treated foliage to untreated plant tissue. All weed species translocated $^{14}C$-ethofumesate to untreated leaf tissue when $^{14}C$-ethofumesate was applied to seedlings at the two-leaf stage. Ethofumesate was moved basipetally to the stem and root components of two-leaf pigweed and lambsquarter seedlings. High percentages of $^{14}C$ complexed with polar constituents in sugarbeet seedlings. The amount of metabolites recovered in the non-polar fraction depended on the stage of plant growth. Total photosynthesis and respiration in pigweed was inhibited 4 hr after foliar application and did not recover. $CO_2$ uptake and evolution were

also inhibited in sugarbeet leaves but they recovered rapidly, depending on age of plant at treatment. The stage of plant development was the key factor in determining species response to foliar treatments of ethofumesate in terms of absorption, metabolism, and total photosynthesis and respiration.

## INTRODUCTION

Successful weed control in sugarbeet or any crop using foliar-applied herbicides depends on the absorption and translocation of the biologically active compound to the site of phytotoxic action in sufficient quantities to kill the weed before metabolism can detoxify the compound. For the chemical to be selective, the crop must either limit uptake or movement from the treated areas, or must enhance deactivation of the potential herbicide.

Ethofumesate is a potential postemergence herbicide for the selective control of broadleaf weeds in sugarbeet and is currently under experimental permit for this purpose (3,7,8). Redroot pigweed and lambsquarter are highly susceptible, and common ragweed moderately susceptible to foliar applications of ethofumesate, depending on stage of growth at treatment (3).

The purpose of this study was to determine the contribution of foliar absorption, translocation, and metabolism to ethofumesate toxicity and selectivity at the two-, four-, and six-leaf stages of plant development of sugarbeet, redroot pigweed, ragweed, and lambsquarter. $CO_2$ uptake and evolution were also studied for sugarbeet and redroot pigweed at two growth stages.

## MATERIALS AND METHODS

$^{14}$C-absorption, translocation, and metabolism. Sugarbeet, common ragweed, redroot pigweed, and lambsquarter were germinated in vermiculite in the greenhouse and transferred to 1/2 strength Hoagland's nutrient solution in foil-wrapped, 250 ml plastic pots with sponge supports. Plants were grown to the desired stage of development (two-, four-, or six-leaf), selected for uniformity, and placed in a growth chamber at 25 C and 27 klux with a spec. act. = 1.35 µCi/µmole) was dissolved in ethanol and additional formulated herbicide added to obtain a concentration approximating the use rate for foliar application, 1.69 kg/ha. A 10 µl drop (0.1 µci) of herbicide was placed in the middle of the upper leaf surface of one of the first two true leaves of each plant species. The leaf was held horizontal until the drop dried, after which the plants were moved back to the growth chamber. Plants for the absorption portion of the study were harvested 3 and 24 hours after treatment. The treated leaf of each plant was rinsed with 20 ml of 50% ethanol to remove any remaining herbicide. The plants were freeze-dried and dissected into treated leaf, other leaves, stem, and root segments. All samples were combusted with a Packard 306 Tri-Carb Oxidizer to determine the quantity of $^{14}$C in each plant part. Released $^{14}CO_2$ was trapped with 12 ml Carbo-Sorb$^{R}$ and further diluted with 10 ml Permaflour$^{R}$ V liquid scintillation solution. The vials containing the sample were radioassayed by liquid scintillation spectrometry and the data expressed as µg ethofumesate absorbed per g dry weight. The treatments were replicated four times in a randomized split block design.

Two replications for radioautography and three for quantitative

determination were harvested 1 and 5 days after treatment for the translocation and metabolism portions of the study. All plants were cultured and treated as previously described. After washing the treated leaf with 50% ethanol, plants were frozen on dry ice, freeze-dried, and either mounted for radioautography (1) or the treated leaf separated from the rest of the plant for dry weight determination and extraction of the two portions. Plant parts were homogenized twice in 50 ml 80% ethanol with a Sorvall Omni-Mixer. After grinding, the plant extracts were filtered through Whatman No. 1 filter paper under vacuum and rinsed with ethanol. The ethanol-insoluble residue was collected for dry weight and combusted for $^{14}C$ determination. The ethanol-soluble filtrate was evaporated in vacuo and the remaining aqueous residue partitioned twice with 40 ml dichloromethane. Both phases (dichloromethane- and water-soluble) were sampled for radioactivity and chromatographic determination of free $^{14}C$-ethofumesate and $^{14}C$-metabolites. All aliquots were separated on 250 μmeter think silica gel G thin layer chromatography plates. The plates were radioautographed and the $^{14}C$-labeled spots on the plate removed and radioassayed. All radioassay data was corrected for background and efficiency determined by channels ratio method.

Photosynthesis study. Four sugarbeet or pigweed plants per 946 ml waxes cups were grown in soil until the plants had reached two different stages of development, two- or six-leaf. The plants were grown in the greenhouse supplemented with artificial lighting to provide a 16 hr day. Temperature ranged from a medium of 20°C at night to a maximum of 33 C during the day. Fourty-eight hours prior to measurement plants were transferred to a growth chamber at 25 C in light of 27 klux with 14 hours of day length. Plants received 28 hr light during this period.

Photosynthesis and respiration measurements were made by placing the cups, one at a time, in a sealed, clear plexi-glass test chamber located in the same growth chamber. The chamber was attached to a Beckman Model IR 215 $CO_2$ infrared gas analyzer. Compressed air for the open flow system was passed through the chamber at a rate of 500 cc per minute. Measurements were made at 0, 4, 24, 48, and 96 hours after the foliar treatments with ethofumesate (2.24 kg/ha) and for comparative purposes, desmedipham (ethyl m-hydroxycarbanilate carbanilate) (0.82 kg/ha). Plants were sprayed in 285 l/ha at 2.11 kg/cm pressure. Leaf area was determined with an automatic area meter (Lambda Instruments).

All data presented are the means of two experiments.

## RESULTS AND DISCUSSION

Absorption of foliar-applied $^{14}C$-ethofumesate by sugarbeet and three weed species was dependent on stage of growth on treatment and time of exposure to the chemical (Table 1). The two susceptible species, lambsquarter and particularly pigweed, absorbed more ethofumesate at both the two- and four-leaf stages of growth than sugarbeet. After 24 hr exposure the moderately susceptible ragweed absorbed more than the tolerant sugarbeet, the differential widening with increasing plant age. A previous report indicated no increase in absorption by sugarbeet after 3 hr exposure to ethofumesate (6). A significant increase in absorption was observed with increased exposure (24 hr) to ethofumesate applied to two-leaf sugarbeet and the three weed species. This difference was not observed for sugarbeet at either the four- or six-leaf stages of growth.

Translocation also appeared to be a significant factor in selectivity.

Regardless of stage of growth at treatment, no significant accumulation of $^{14}C$ was observed in the untreated plant segments of sugarbeet 24 hr after foliar application (Figure 1 and Table 2). However, all weed species translocated $^{14}C$-ethofumesate to untreated leaf tissue (acropetal movement) when applications of ethofumesate were made at the two-leaf stage of development (Figures 2 to 4 and Table 2). Basipetal movement of $^{14}C$ to the stem and root components was detected only in two- and four-leaf pigweed and two-leaf lambsquarter species. A similar distribution pattern was observed 3 hr after application. The absorption and translocation data appeared highly consistent with the field information on selectivity of foliar-applied ethofumesate to sugarbeet and associated weed species at the three leaf stages of growth (2).

Few species differences were observed in the amount of $^{14}C$- ethofumesate (dichloromethane-soluble) recovered at any one stage of growth or time of exposure (Tables 3, 4, and 5). After 5 days of exposure to ethofumesate applied at the four- and six-leaf stages, a significant increase in $^{14}C$-metabolites was detected in all species, but especially in the weed species at the six-leaf stage. However, as much as 91% of the total extractable $^{14}C$ in sugarbeet was found in the water-soluble residue. This percentage increased markedly from plants treated at the two-leaf (59.9% after 5 days) to those treated at the four-leaf stage (91.2%), with no additional increase detected in plants treated at the six-leaf stage (90.3%). Complexing of parent ethofumesate and metabolites with water-soluble plant constituents in sugarbeet leaves was a significant factor in the detoxication process of root-applied $^{14}C$-ethofumesate (4). Thus, the same mechanism appeared likely with foliar-applied ethofumesate on sugarbeet, particularly at the four- and six-leaf stages of

growth. Inactivation of ethofumesate by the weed species involved, to a greater extent, breakdown of ethofumesate to organic-soluble metabolites. The amount of dichloromethane-soluble $^{14}C$ found in sugarbeet treated at the four- and six-leaf stages was extremely small considering less than 10% of the total was recovered in this fraction. For both stages, less than 40% was detected as metabolite. Therefore, the nature of the metabolism appears to be different between the tolerant sugarbeet and the three weed species.

Ethofumesate applications reduced the $CO_2$ uptake of sugarbeet and pigweed within 4 hr after treatment at both the two- and six-leaf stages (Table 6). Total photosynthesis in pigweed did not recover within the 96 hr observation period for either growth stage. The six-leaf sugarbeet recovered rapidly, but 96 hr were required to detect significant recovery in sugarbeet treated at the two-leaf stage. Dark respiration of ethofumesate-treated sugarbeet was reduced when applied to the two-leaf but not the six-leaf stage of growth. For pigweed, $CO_2$ evolution was reduced regardless of stage of growth at treatment. Regarding recovery, dark respiration paralleled photosynthetic activity. Desmedipham, reported to be an effective photosynthetic and respiratory inhibitor (7), was included in this study for comparison. This data confirms previous results obtained with foliar-applied desmedipham and, in terms of selectivity, indicates similarity to ethofumesate.

Ethofumesate appears to be a rapid inhibitor of respiration and photosynthesis. The inhibition followed by recovery of sugarbeet, but not pigweed, indicates that metabolism of ethofumesate is involved in selectivity. At the two-leaf stage of sugarbeet where photosynthetic recovery was not apparent for 4 days (Table 6), metabolism of parent

ethofumesate was also slow (Table 3). However, recovery of $CO_2$ uptake was rapid (within 24 hr) at the six-leaf stage (Table 6) where 85% of extractable $^{14}C$ was complexed with polar plant constituents after 24 hr exposure to $^{14}C$-ethofumesate (Table 5).

Several factors may have contributed to the observed selectivity to foliar applications of ethofumesate. For the youngest of seedlings, rapid and continued absorption, extensive accumulation in untreated plant components, particularly leaves, slow metabolism, and inhibited photosynthesis all appeared to play an active role in susceptibility of redroot pigweed and lambsquarter to foliar applications of ethofumesate. The stage of plant development was the primary factor in determining species response to ethofumesate treatment relative to absorption, translocation, metabolism, and $CO_2$ uptake and evolution.

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Table 1. Absorption of foliar applied $^{14}$C-ethofumesate by sugarbeet and three weed species as influenced by stage of growth at application and time of exposure.

| Species | Exposure time (hr) | Stage of growth at treatment: two-leaf | four-leaf | six-leaf |
|---|---|---|---|---|
| | | (µg/g dry weight) | | |
| Sugarbeet | 3 | 52.4 b-e[a] | 2.4 a | 1.0 a |
| | 24 | 151.6 gh | 13.5 abc | 7.6 ab |
| Ragweed | 3 | 196.0 h | 50.2 a-e | 55.5 b-e |
| | 24 | 346.0 j | 67.7 de | 61.1 cde |
| Pigweed | 3 | 398.1 k | 293.6 i | 19.2 a-d |
| | 24 | 936.9 m | 558.7 l | 55.2 b-e |
| Lambsquarter | 3 | 444.9 k | 74.4 ef | 10.1 ab |
| | 24 | 540.4 l | 301.3 ij | 121.0 fg |

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

Table 2. Distribution of $^{14}C$-ethofumesate 24 hr after foliar application to sugarbeet and three weed species as influenced by stage of growth at application.

| Species | Stage of growth (leaves) | Plant parts: Treated leaf | other leaves | Stem | Root |
|---|---|---|---|---|---|
| | | (µg/g dry weight) | | | |
| Sugarbeet | 2 | 138.2 b[a] | 8.0 a | 3.5 a | 2.2 a |
| | 4 | 11.9 a | 0.2 a | 0.8 a | 0.7 a |
| | 6 | 6.9 a | 0.2 a | 0.2 a | 0.3 a |
| Ragweed | 2 | 228.8 c | 87.5 b | 17.4 b | 14.0 a |
| | 4 | 50.9 a | 15.8 a | 1.0 a | 1.9 a |
| | 6 | 50.1 a | 9.0 a | 1.3 a | 1.8 a |
| Pigweed | 2 | 373.0 e | 368.8 d | 85.6 b | 110.0 b |
| | 4 | 320.5 d | 189.2 c | 30.3 a | 19.0 a |
| | 6 | 28.5 a | 12.8 a | 13.4 a | 2.0 a |
| Lambsquarter | 2 | 383.0 e | 119.5 b | 21.0 a | 17.6 a |
| | 4 | 247.1 c | 31.0 a | 11.5 a | 12.6 a |
| | 6 | 113.9 b | 5.0 a | 1.4 a | 2.5 a |

[a]Means within columns having identical letters are not significantly different at the 5% level using Duncan's multiple range test.

Table 3. Metabolism of foliar applied $^{14}$C-ethofumesate to four plant species at the two-leaf stage of growth.

| Days after treatment | Species | Plant part[a] | Dichloromethane-soluble | | | | Water-soluble residue (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | Metabolites (%) | | Unmetabolized ethofumesate (%) | | | |
| 1 | Sugarbeet | Treated leaf | 4.0 | a[b] | 94.4 | a | 40.0 | cd |
| | | Other | 6.6 | a | 92.0 | a | 34.6 | cd |
| | Ragweed | Treated leaf | 8.9 | a | 90.9 | a | 12.0 | ab |
| | | Other | 7.0 | a | 91.9 | a | 11.5 | ab |
| | Pigweed | Treated leaf | 1.2 | a | 97.2 | a | 25.7 | bc |
| | | Other | 3.2 | a | 95.5 | a | 14.0 | ab |
| | Lambsquarter | Treated leaf | 6.8 | a | 91.9 | a | 16.6 | ab |
| | | Other | 9.0 | a | 90.3 | a | 9.5 | a |
| 5 | Sugarbeet | Treated leaf | 4.5 | a | 94.0 | a | 59.9 | e |
| | | Other | 4.9 | a | 93.1 | a | 48.8 | de |
| | Ragweed | Treated leaf | 10.1 | a | 89.8 | a | 14.9 | ab |
| | | Other | 7.2 | a | 91.2 | a | 7.7 | a |
| | Pigweed | Treated leaf | 3.1 | a | 95.3 | a | 19.9 | a |
| | | Other | 6.0 | a | 94.0 | a | 16.0 | ab |
| | Lambsquarter | Treated leaf | 10.0 | a | 88.6 | a | 10.8 | ab |
| | | Other | 11.3 | a | 88.0 | a | 7.3 | a |

[a]Other - remaining leaves, stem, and root portions.

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

Table 4. Metabolism of foliar applied $^{14}$C-ethofumesate to four plant species at the four-leaf stage of growth.

| Days after treatment | Species | Plant part[a] | Dichloromethane-soluble: Metabolites (%) | Dichloromethane-soluble: Unmetabolized ethofumesate (%) | Water-soluble residue (%) |
|---|---|---|---|---|---|
| 1 | Sugarbeet | Treated leaf | 13.0 abc[b] | 87.0 e | 79.4 e |
| | | Other | ND[c] | ND | ND |
| | Ragweed | Treated leaf | 6.0 ab | 93.0 e | 31.7 abc |
| | | Other | 10.9 ab | 88.9 e | 30.8 abc |
| | Pigweed | Treated leaf | 12.0 abc | 86.2 e | 52.2 d |
| | | Other | 23.2 c-f | 73.7 bcd | 29.9 abc |
| | Lambsquarter | Treated leaf | 4.0 a | 91.0 e | 40.9 cd |
| | | Other | 12.1 abc | 85.1 de | 31.6 abc |
| 5 | Sugarbeet | Treated leaf | 33.3 f | 72.9 bc | 91.2 e |
| | | Other | ND | ND | ND |
| | Ragweed | Treated leaf | 15.0 a-d | 83.0 cde | 36.4 bc |
| | | Other | 17.2 b-e | 81.2 b-e | 19.7 a |
| | Pigweed | Treated leaf | 27.9 ef | 70.0 b | 30.9 abc |
| | | Other | 29.1 ef | 55.9 a | 19.8 a |
| | Lambsquarter | Treated leaf | 30.0 f | 69.3 b | 25.4 ab |
| | | Other | 25.3 def | 70.6 b | 19.0 a |

[a]Other = remaining leaves, stem, and root portions.

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

[c]ND = non-detectable.

Table 5. Metabolism of foliar applied $^{14}$C-ethofumesate to four plant species at the six-leaf stage of growth.

| Days after treatment | Species | Plant part[a] | Dichloromethane-soluble: Metabolites (%) | | Dichloromethane-soluble: Unmetabolized ethofumesate (%) | | Water-soluble residue (%) | |
|---|---|---|---|---|---|---|---|---|
| 1 | Sugarbeet | Treated leaf | 13.9 | a[b] | 81.2 | e | 85.0 | f |
| | | Other | ND[c] | | ND | | ND | |
| | Ragweed | Treated leaf | 28.2 | cde | 68.0 | bcd | 34.0 | bcd |
| | | Other | 27.0 | b-e | 69.9 | b-e | 39.3 | cde |
| | Pigweed | Treated leaf | 22.1 | a-d | 72.4 | cde | 52.9 | e |
| | | Other | 15.9 | ab | 77.7 | de | 30.8 | abc |
| | Lambsquarter | Treated leaf | 16.0 | ab | 79.9 | de | 46.9 | de |
| | | Other | 14.1 | a | 80.8 | e | 41.2 | cde |
| 5 | Sugarbeet | Treated leaf | 38.2 | e | 60.8 | bc | 90.3 | f |
| | | Other | ND | | ND | | ND | |
| | Ragweed | Treated leaf | 51.9 | fg | 42.0 | a | 24.0 | ab |
| | | Other | 55.5 | g | 40.0 | a | 35.2 | bcd |
| | Pigweed | Treated leaf | 38.3 | ef | 59.1 | b | 40.1 | cde |
| | | Other | 50.2 | fg | 47.1 | a | 18.1 | a |
| | Lambsquarter | Treated leaf | 56.0 | g | 39.2 | a | 23.9 | ab |
| | | Other | 59.6 | g | 35.5 | a | 26.4 | abc |

[a]Other = remaining leaves, stem, and root portions.

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

[c]ND = non-detectable.

Table 6. Percent of initial total photosynthesis of two species influenced by postemergence herbicide application and stage of growth at treatment.

| Stage of growth (leaves) | Herbicide Species | Time after treatment (hr) 4 | 24 | 48 | 96 |
|---|---|---|---|---|---|
| | | (% of initial photosynthetic rate) | | | |
| 2 | Ethofumesate | | | | |
| | Sugarbeet | 52*[b] fgh[a] | 34 c-f | 30 b-e | 67* hi |
| | Redroot pigweed | 54* ghi | 20 a-e | 18 a-d | 22* a-e |
| | Desmedipham | | | | |
| | Sugarbeet | 31* b-e | 63 hi | 66 hi | 126* m |
| | Redroot pigweed | 18* a-d | 9 a | 7 a | 14* ab |
| 6 | Ethofumesate | | | | |
| | Sugarbeet | 64* hi | 98 kl | 93 kl | 108 klm |
| | Redroot pigweed | 73* ij | 39 efg | 24 a-e | 15* abc |
| | Desmedipham | | | | |
| | Sugarbeet | 62* hi | 64 hi | 90 jk | 112 lm |
| | Redroot pigweed | 37* d-g | 8 a | 7 a | 6* a |

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

[b]Asterisks indicate significance between the amount of photosynthesis prior to and 4 hr or 96 hr after herbicide treatment using students "T" test.

Table 7. Percent of initial dark respiration of two species as influenced by postemergence herbicide application and stage of growth at treatment.

| Stage of growth (leaves) | Herbicide Species | Time after treatment (hr) 4 (% of initial respiration rate) | 24 | 48 | 96 |
|---|---|---|---|---|---|
| 2 | Ethofumesate | | | | |
| | Sugarbeet | 42*[b] def[a] | 31 bcd | 38 cde | 67* ghi |
| | Redroot pigweed | 75* hij | 22 abc | 9 a | 11* a |
| | Desmedipham | | | | |
| | Sugarbeet | 48* d-g | 64 ghi | 90 jk | 100* kl |
| | Redroot pigweed | 39* c-f | 22 abc | 15 ab | 15* ab |
| 6 | Ethofumesate | | | | |
| | Sugarbeet | 80 ij | 119 lm | 140 n | 143* n |
| | Redroot pigweed | 78* ij | 67 ghi | 42 def | 42* def |
| | Desmedipham | | | | |
| | Sugarbeet | 64* ghi | 62 ghi | 89 jk | 112 lm |
| | Redroot pigweed | 91 jk | 88 jk | 55 efg | 58* fgh |

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

[b]Asterisks indicate significance between the amount of photosynthesis prior to and 4 hr or 96 hr after herbicide treatment using students "T" test.

Figure 1. Translocation of $^{14}C$-ethofumesate in sugarbeet. The radio-autographs (A) at the two-, four-, and six-leaf stages of growth (left to right) and corresponding plant specimens (B). Arrows indicate treated leaves.


A

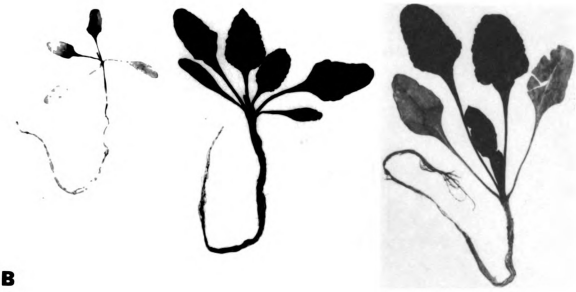
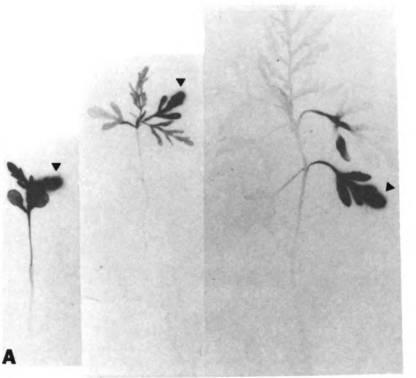

B

Figure 2. Translocation of $^{14}C$-ethofumesate in common ragweed. The radioautographs (A) at the two-, four-, and six-leaf stages of growth (left to right) and corresponding plant specimens (B). Arrows indicate treated leaves.


A

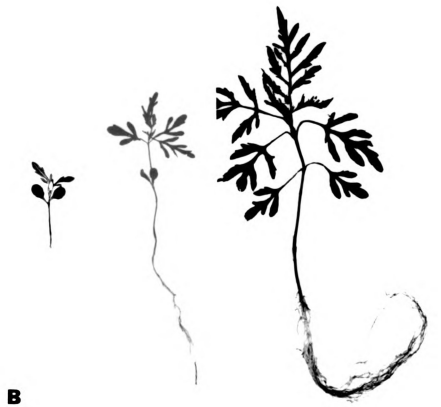
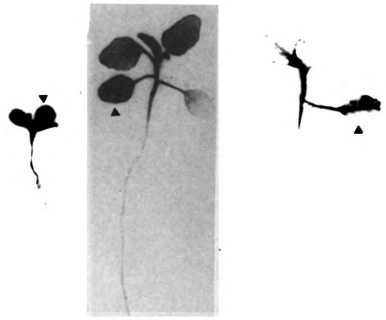

B

Figure 3. Translocation of $^{14}C$-ethofumesate in redroot pigweed. The radioautographs (A) at the two-, four-, and six-leaf stages of growth (left to right) and corresponding plant specimens (B). Arrows indicate treated leaves.



A

B

Figure 4. Translocation of $^{14}C$-ethofumesate in common lambsquarter. The radioautographs (A) at the two-, four-, and six-leaf stages of growth (left to right) and corresponding plant specimens (B). Arrows indicate treated leaves.

A

B

# CHAPTER 5

## SUMMARY AND CONCLUSIONS

Studies were conducted in the field to evaluate the various factors influencing ethofumesate and other postemergence herbicide treatments on sugarbeet. Greenhouse and laboratory studies were initiated to determine the basis for the observed interaction of sequential herbicide combinations in the field, and to study the physiological bases for sugarbeet tolerance to soil- and foliar-applied ethofumesate.

Preemergence application of the combination of ethofumesate plus TCA caused greater suppression than pyrazon plus TCA when followed by a postemergence herbicide treatment to field grown sugarbeets. Additionally, sugarbeet tolerance and weed control depended largely on the stage of plant growth at time of foliar application. Foliar treatment at the two- to four-leaf stage provided minimal sugarbeet injury with optimal weed control results. Depending on temperature and light intensity subsequent to foliar application of herbicides, time-of-day at application may also play an important role in determining the degree of selectivity to ethofumesate. On a cloudless day where the ambient temperature reached 32 C, mid-afternoon treatment combinations were less phytotoxic and provided equal or greater weed control compared to morning or evening treatments.

Exposure of sugarbeet to ethofumesate and TCA decreased the

epicuticular wax deposition on the leaf surfaces. Greater absorption of $^{14}C$-herbicides was observed in plants that received preemergence treatments of ethofumesate and TCA as compared to pyrazon or a control. Therefore, the basis for the interaction of sequential herbicide combinations was increased absorption of foliar applications due to reduction in epicuticular leaf wax deposition from soil-applied herbicides.

The basis for selectivity of ethofumesate in sugarbeet and associated weed species varied with manner of application. For root-applied ethofumesate, slow translocation to the leaves and rapid metabolism within sugarbeet and ragweed compared to pigweed and lambsquarter appeared related to species differences in phytotoxicity. Absorption, translocation, and metabolism all contributed to the observed selectivity of foliar applications of ethofumesate. As with the field study, response to foliar treatment varied primarily with stage of plant development. For the youngest seedlings, rapid and continued absorption, extensive accumulation in untreated plant components, particularly leaves, and slow metabolism all played an active role in susceptibility of pigweed and lambsquarter to foliar applications of ethofumesate. Stage of growth also determined species response to photosynthesis after foliar treatment with ethofumesate. The implication, therefore, is that both applied and basic oriented researchers must remain cognizant of potential differences in response to treatment of plants in varied stages of morphological and physiological growth.

In conclusion, it is apparent from these studies why variations exist in selectivity of ethofumesate in sugarbeet. The preplant or preemergence herbicide used, stage of growth of sugarbeet and weed species at treatment, and environmental factors should all be considered when

selecting the specific foliar herbicide treatment. Obviously, the weed population is also an important consideration. The time is past for the "spray and pray" approach to weed control.

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