SELECTIVITY OF PHENMEDIPHAM (METHYL *M*-HYDROXYCARBANILATE *M*-METHYLCARBANILATE) AND ETHYL *M*-HYDROXYCARBANILATE CARBANILATE IN SUGAR BEET

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Selectivity of Phenmedipham (Methyl M-Hydroxycarbanilate M-Methylcarbanilate) and Ethyl M-Hydroxycarbanilate Carbanilate in Sugar Beet

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Larry Wayman Hendrick

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ABSTRACT

SELECTIVITY OF PHENMEDIPHAM (METHYL M-HYDROXYCARBANILATE
M-METHYLCARBANILATE) AND ETHYL M-HYDROXYCARBANILATE
CARBANILATE IN SUGAR BEET

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Phenmedipham (methyl m-hydroxycarbanilate m-methylcarbanilate) and EP-475 (ethyl m-hydroxycarbanilate carbanilate) were applied to sugar beet (Beta vulgaris L.) locations in different years. Broadleaf weed control was obtained at rates of 1.12 to 1.68 kg/ha (1 to 1.5 lb/A) or slightly less when in combination with other herbicides. No herbicidal treatment reduced root yield or the sugar content, even when a high level of foliar inhibition was observed. EP-475 must be included as a postemergence treatment to obtain effective redroot pigweed (Amaranthus retroflexus L.) control. Studies in the laboratory were conducted to observe the effect of phenmedipham and EP-475 on photosynthesis of wild mustard [Brassica kaber (DC.) L.C. Wheeler "pinnatifida" (Stokes) L.C. Wheeler], redroot pigweed, and sugar beet. Sugar beet recovered from the initial inhibition of photosynthesis due to herbicide application but wild mustard did not. Photosynthesis in redroot pigweed was permanently inhibited by EP-475 but was temporarily inhibited by phenmedipham. Further laboratory studies were conducted to determine the basis for selectivity of these two herbicides in these three species

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by evaluating spray retention, absorption, translocation, and metabolism. Selectivity can not be explained by differences in spray retention or absorption. Only acropetal translocation of this herbicide was observed. Redroot pigweed translocated more EP-475 than phenmedipham. Sugar beet metabolized both herbicides quickly but wild mustard did not. Redroot pigweed metabolized phenmedipham quickly but metabolized EP-475 slowly. This difference in metabolism was the difference in the rate of the initial detoxication reaction involving the parent herbicide.

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INTRODUCTION

It has been shown that weeds have the potential to harm crop plants and thus cause economic loss (5, 31, 41, 43). In Michigan sugar beets, redroot pigweed is a major annual broadleaf weed problem.

A new postemergence herbicide, EP-475 (ethyl _m_-hydroxycarbanilate carbanilate) has been reported to kill redroot pigweed[1] (1, 9, 26). Phenmedipham (methyl _m_-hydroxycarbanilate _m_-methylcarbanilate) is an analog of EP-475 that exhibits little or no activity on redroot pigweed. The purpose of this research was to study the use and selectivity of EP-475 and phenmedipham in sugar beets.

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[1]Dawson, J. H. 1971. Effects of herbicides on sugar beets. Weed Sci. Soc. Amer. Abstr. No. 3.

CHAPTER 1

Carbanilate and Related Herbicides

Weeds and Crops

Under a noncompetitive situation, one redroot pigweed plant can produce 229,000 seeds and wild mustard can produce 1,200 to 2,700 seeds per plant (35, 22). But when there were 60 redroot pigweed plants per 0.84 sq m, 5,200 viable seeds were produced per plant (35).

Brimhall, et al. (5) found that redroot pigweed densities of one per eight sugar beets or greater reduced the root weight of beet roots and beet tops but did not affect percentage sugar. This reduction in yield was attributed to competition for light. On a plant basis, broadleaf weeds reduced sugar beet root yield more than grass weeds (5, 43).

Weatherspoon and Schweizer (41) found a significant reduction in root yield and total pounds of sucrose per acre with only one kochia (*Kochia scoparia* (L) Schrader) plant per 7.5 meters of sugar beet row. Percent sucrose was significantly reduced only when there was one kochia plant or more per 30.5 cm of row.

Miller[1] found that any density of weeds used reduced

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[1]Miller, J. R. 1963. An evaluation of competition between weeds and sugar beets (*Beta vulgaris* L.). Ph.D. Thesis. Michigan State University, East Lansing. 85p.

sugar beet yield if the weeds remained in the beets longer than 4 weeks. The percent sucrose of the harvested root was not affected by weed competition.

Shadbolt and Holm (31) showed a loss in yield in vegetable row crops due to present of redroot pigweed and water hemp (*Acnida* sp.).

Efficacy of Phenmedipham and EP-475 in Sugar Beets

Arndt and Kotter (1) showed that postemergence application of phenmedipham was more selective in cotyledonary sugar beets and weeds at 0.25 kg/ha, and in older sugar beets at 0.50 kg/ha or more, on common chickweed (*Stellaria media* (L.) Cyrill), (*Senecio vulgaris* L.), common lambsquarters (*Chenopodium album* L.), henbit (*Lamium amplexicaule* L.), white mustard (*Sinapis alba*, Grey), and galinsoga (*Galinsoga parviflora*, Cav), knapweed (*Centaurea cyanus* L.), bedstraw (*Galium aparine* L.), and (*Alopecurus myosuroides* Huds). Redroot pigweed at these growth stages showed little susceptibility at these rates but showed susceptibility at rates injurious to the sugar beet.

Dexter (9), studying various herbicides used for weed control in sugar beet, found that no individual herbicide gave good broad spectrum weed control unless postemergence applications of phenmedipham or pyrazon (5-amino-4-chloro-2-phenyl-3 (2H)-pyridazinone) plus dalapon (2,2-dichloropropionic acid) followed a preemergence or preplant incorporated treatment. Initial sugar beet injury increased with the use of combinations but no yield reductions resulted.

Dawson[2] found that sugar beets were tolerant of phenmedipham but were injured under certain unfavorable weather conditions. However, injury was temporary and the sugar beets recovered.

Schweizer and Weatherspoon (30) found that phenmedipham did not satisfactorily control pigweed seedlings that had two to five leaves but was controlled by EP-475. EP-475 may injure sugar beets more than phenmedipham but sugar beet tolerance and weed control may be improved by a mixture of phenmedipham plus EP-475.

Edwards (11) applied Schering formulation No. 4075 containing 16.7 percent (w/w) phenmedipham at 1.12 kg/ha on red beets and obtained good control of most annual broadleaved weeds in very young stages. A spray volume of 187 L/ha gave better weed control than other volumes used.

Holmes (17) also applied Schering formulation No. 4075 at 1.12 kg/ha and found that it gave postemergence control of most important annual broadleaved weeds except prostrate knotweed (Polygonum aviculare L.) in sugar beets and did not affect crop stand, yield of roots, or sugar content.

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[2]Dawson, J. H. 1971. Effects of herbicides on sugar beets. Weed Sci. Soc. Amer. Abstr. No. 3.

Miller and Nalewaja (26) applied phenmedipham with various additives in the field and greenhouse and found that nonphytotoxic oils were more effective than surfactants as additives to phenmedipham on green foxtail (Setaria virdis (L) Beauv.), yellow foxtail (Setaria glauca (L.) Beauv.), redroot pigweed, or common lambsquarters. No additive used enhanced herbicidal activity on kochia or wild mustard. Linseed oil reduced the herbicidal activity of phenmedipham on kochia.

Van der Zweep[3], *et al*. measured $^{14}CO_2$ assimilation in sugar beet as affected by single drop applications of phenmedipham solutions. They detected a local inhibition of photosynthesis and no systemic effect and concluded that uniform foliar coverage was necessary to kill susceptible plants.

Phenmedipham and Soils

Kossman[4] considered uptake from soil of phenmedipham to be insignificant due to the chemical's inherent properties of strong adsorption in upper layer of soil, its slight water solubility (3 ppm), and its high sensitivity

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[3]Van der Zweep, W., J. L. P. Van Oorschot, and A. Reisler. 1969. Laboratory studies on the selectivity of phenmedipham. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Schering AG. 1969. p. 10-11.

[4]Kossman, K. 1969. Phenmedipham residues in plant and soil. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Schering AG. 1969. p. 16-20.

to hydrolyzing agents. He also found that phenmedipham is continuously decomposed in the slightly acid soils of low organic matter content used for beet production (23). Half-lives of 28 to 55 days were recorded, depending on experimental conditions and soil type. In the field the formulated herbicide penetrated only very little beyond a depth of 12.7 cm.

Sonawane and Knowles (33) examined decomposition of phenmedipham and *m*-aminophenol in alkaline soil and found that phenmedipham is hydrolyzed to *m*-aminophenol via methyl *m*-hydroxycarbanilate. In the soil the *m*-methylphenol can undergo physical adsorption and chemical complexing with soil components.

Effect of Phenmedipham on Photosynthesis

El-Sharkawy and Hesketh (12) measured the rate of CO_2 fixation in several species and found that pigweed had twice the fixation rate of sugar beet and a fixation rate equal to that of corn (*Zea mays* L. "Funks G711A"), bermudagrass (*Cynodon lactylon* (L.) Pers. "Coastal") and grain sorghum (*Sorghum vulgare* L. "Hegari"). The redroot pigweed had similar leaf anatomical characteristics to the above tropical grasses.

Chen, *et al*. (7) presented data that supports the hypothesis that many high photosynthetic capacity plants, which also includes many crop plants, are very competitive plants and often become serious weeds. Usually plants such as redroot pigweed with high photosynthetic CO_2 fixation rates have a low CO_2 compensation concentration.

Good (14) noted that the atomic configuration common to all Hill reaction inhibitors may be represented by

$$R'-\underset{\substack{|\\H}}{N}-\underset{\substack{\|\\X}}{C}-R''$$

where X represents a nitrogen or oxygen atom. The nitrogen must be attached to a group of considerable size, but it must not sterically alter the activity of the nitrogen.

Van Overbeek (39) noted that although s-triazines, phenylureas, and acylanilides have similar biological actions, they are dissimilar in structure. Their biological activity must not be due to covalent bond reactions. As also noted by Good above, the similarity these classes of herbicides have must be responsible for their biological activity, obtained by hydrogen bonding of the common NH group and C=O or C=N groups to protein of an enyzme involved in oxidation of water.

Shaw (32) found a high degree of correlation between molecular configuration and herbicidal activity of various carbamates.

Kotter and Arndt (24) found that phenmedipham causes a rapid decrease of CO_2 assimilation in both resistant and susceptible plants, but a resistant plant such as sugar beet soon recovers. Dark respiration of sugar beet was inhibited later in time than was CO_2 assimilation. Dark respiration of sugar beet was inhibited less than that of mustard. The latter was permanently affected but sugar beet recovered.

Arndt and Kotter (1) used starch content as an indicator of the photosynthesis rate of wild mustard and sugar beet as influenced by treatment of phenmedipham. He found that the starch content of wild mustard dropped greatly 24 hours after treatment. In isolated chloroplasts they found no difference in the inhibition of the Hill reaction of a sensitive plant compared to a resistant specie. By using an infrared CO_2 gas analyzer to measure photosynthesis in intact plants, they found that phenmedipham suppressed CO_2 assimilation in sugar beet and weeds but sugar beet and redroot pigweed completely recovered and the other weeds did not. The rapid recovery of sugar beet and redroot pigweed after treatment was attributed to the possibility of rapid metabolism or difference in absorption.

Willenbrink[5] found a 90 percent inhibition of the photosynthetic rate in intact wild mustard (*Sinapis arvensis* L.) with phenmedipham. Further metabolic experiments indicated that during photosynthesis it is the Hill reaction which is inhibited by phenmedipham.

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[5]Willenbrink, J. 1969. The action of phenmedipham on the CO_2 fixation during photosynthesis in young sugar beet leaves. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Schering AG. 1969. p. 7-9.

Factors for Selectivity of Herbicides

Buchel (6) stated that all the work with chloroplasts on mechanism and maximum activity had no relation to the selectivity of herbicides. He went on to say that a selective herbicide is the result of an empirical long range field testing and is more a statistical event than a result of scientific design. He believed that the work with chloroplasts does not help significantly and that more fundamental plant physiology and experiments with intact species are necessary.

Robertson and Kirkwood (28) stated that if the effectiveness of a herbicide is dependent on the concentration of the chemical reaching the site of action, then any factor that affects the rate of accumulation at that site must be considered in the overall view of the mode of action of the herbicide.

A. Retention of Herbicides on Leaves

Hibbitt (16) used wild oat (*Avena fatua* L.) and flax (*Linum usitatissimum* L.) to study retention of sprays on leaf surfaces. With growth of wild oat, an increase in amount of spray retained per plant and per unit weight of plant occurred. A constant volume of spray per plant was retained on flax as it grew, related to a decrease in projected surface area which is exposed to spray as a proportion of the whole. Also, cotyledons retain greater amounts of spray per unit weight than true leaves.

B. Absorption and Translocation of Herbicides

Robertson and Kirkwood (29) reviewed several factors influencing absorption of herbicides. Mature leaves absorb less than expanding leaves due to the thick cuticles. Rapid penetration is possible through the thin cuticle at veins and the midrib. Higher moisture conditions will swell the hydrophilic groups of the cuticle, pushing the apolar wax units apart promoting apoplastic absorption of water-soluble herbicides. The heavy esters of 2,4-D (2,4-dichlorophenoxyacetic acid) are absorbed greater than other formulations of 2,4-D but must be converted to the acid moiety before translocation.

Initial studies[6] indicated that very little leaf-applied phenmedipham could be found in the roots of sugar beet or in the top and root residue even 28 days after application. Phenmedipham content in sugar beet tops reached a maximum at 14 days after application.

Bischof, et al. (3) found that high light intensity increased the phenmedipham content in sugar beet and white mustard (*Sinapis alba*, Grey). Sugar beet was damaged at temperatures of 30 and 40 C but the decline of phenmedipham content in both beets and mustard was quicker at

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[6]Jenny, N. Personal communication. Nor-Am Agricultural Chemicals, Woodstock, Illinois.

higher temperatures than at lower temperatures. Both sugar beet and mustard absorb phenmedipham from nutrient solutions and from foliar application in similar amounts. One hour after leaf application, the largest portion of the absorbed compound had already penetrated, which was only a small portion of that applied. Greatest penetration occurred when the sugar beet and mustard had full cotyledonary leaves and one-half formed first true leaves.

C. Metabolism of Carbamates

Kearney (20) isolated and characterized an enzyme obtained from *Pseudomonas* sp. that will hydrolyze carbanilate compounds to aniline and alcohol analogs. It also exhibits amidase activity as it will hydrolyze some acylanilides but not phenylureas.

James and Prendeville (18) used smartweed (*Polygonum lapathifolium* L.), redroot pigweed, and tomato (*Lycopersicum esculentum* Mill.) to study metabolism of chlorpropham (isopropyl *m*-chlorocarbanilate). They found a similar conversion to B-glucosides in all species. There was no modification of the aromatic ring, hydrolysis of the ester linkage, or conversion to an N-hydroxy derivative. They projected that the isopropyl portion must be oxidized at the $-CH_3$ group to $-CH_2OH$, thus glycoside formation could occur readily.

Still (36) examined metabolism of root applied propanil (3',4'-dichloropropionanilide) in rice (*Oryza* sativa L.) plants and found that the parent compound is degraded to 3,4-dichloroaniline and propionic acid. The 3,4-dichloroaniline was first complexed as N-(3,4-dichlorophenyl)-glucosylamine, and then to other unidentified complexes.

Yih (42) used foliar applied propanil on rice plants to investigate metabolism of the herbicide, finding similar results as Still (37). But he found that the aniline-carbohydrate complexes were minor and that the major portion of the 3,4-dichloroaniline is complexed to lignin. The parent compound did not complex to lignin.

Mann, et al. (25) found that carbanilates inhibited amino acid utilization by susceptible species, occurring very soon after contact of the herbicide with plant tissue. He noted that carbamate herbicides and proteins have a common structure, the peptide bond.

Chin, et al. (8) studied metabolism of swep (methyl 3,4-dichlorocarbanilate) in 4-week old rice plants, finding a stable swep-lignin metabolite in the straw, hulls, and bran, but no residue in the endosperm.

Still and Mansager (37) studied metabolism of root-applied chlorpropham in soybean (Glycine max (L.) Merr.) plants and found there was acropetal translocation in these plants and that the carbanilate bond was not broken in metabolism. The polar metabolites of chlorpropham were not translocated once formed in the root or shoot. They suggested that the chlorpropham phenyl nucleus was hydroxylated or oxidized.

Studies conducted by Dittert and Higuchi (10) on hydrolysis of carbamates in alkaline solutions strongly suggested that initial cleavage would be expected to occur at the ester bond. The rate of hydrolysis would depend upon the degree of substitution of the nitrogen.

Bordeleau and Bartha (4) found that azobenzene residues can be formed from variously substituted aniline degradation products of aniline based herbicides in soil.

Bartha and Pramer (2) established that propanil decomposes in soil to carbon dioxide and 3,4-dichloro-aniline, and the latter forms 3,3',4,4'-tetrachloroazo-benzene. All reactions involved soil microorganisms.

D. Metabolism of Phenmedipham and EP-475

Sonawane and Knowles (34) administered EP-475 and phenmedipham orally to white rats and studied the metabolism of these two compounds excreted in urine. They found that both chemicals were rapidly metabolized to ethyl m-hydroxycarbanilate and methyl m-hydroxycarbanilate, respectively. Both metabolites subsequently went to m-aminophenol, and then were N-acetylated to form 3'hydroxy-acetanilide. All of these metabolites were found complexed to natural plant components such as glucuronic acid.

Kotter (10) found that phenmedipham at 3.3×10^{-5} M had no influence on the enzymes catalase, peroxidase, and transaminase *in vitro*, and slight inhibition on glutamate dehydrogenase. There was no effect on citric acid cycle enzymes. There was a strong affinity between phenmedipham and chloroplast membranes, and some adsorption by the chloroplasts was found.

Kassebeer (11) found that a larger amount of phenmedipham entered the foliage of sugar beet and various weeds within the first 4 hours after application. Eight hours after treatment, translocation of phenmedipham within the plant was observed, moving mainly in the direction of the transpiration stream. A very rapid distribution of the compound was observed in wild mustard. Also 8 hours after treatment the beets had decomposed 20 to 40 percent of the compound while the weeds used decomposed only traces of it. All the plants used, except sugar beet, decomposed most of the phenmedipham into methyl *m*-hydroxycarbanilate and into *m*-methylaniline, which in turn can combine with water soluble compounds in plants. Sugar beets decomposed phenmedipham in a different manner than the weeds. In another study, Kassebeer (19) pointed out that even though leaves of some plants are killed, the plant may survive due to a protected growing point and lack of basipetal translocation of phenmedipham. He found that sugar beet rapidly absorbed phenmedipham but metabolized it rapidly, as did redroot pigweed, but with slightly slower metabolism. Some susceptible species had comparably slower metabolism and also slower absorption of phenmedipham. It was concluded that there was no single path of metabolism to account for selectivity but rather the plant species inactivated phenmedipham in different ways. Neither uptake nor rate of degradation alone explained the selectivity of phenmedipham on species tested. There was a straight relationship between susceptibility of various species and their content of parent material since resistant species have a lower and susceptible species have a higher content of active ingredient, which is a function of both absorption and metabolism.

Knowles and Sonawane (21) investigated the fate of foliar applied EP-475 in sugar beet over a 90-day period, and found that the amount of parent compound decreased and decomposition products increased during this time. The major metabolite was ethyl _m_-hydroxycarbanilate. Also, smaller amounts of _m_-aminophenol and very polar compounds were evident.

Looking at possible breakdown products of phenmedipham, and of EP-475 due to structural similarities, three different reactions could be expected as the first degradation steps, as typical of methyl- and phenylcarbamates (15). These are:

(1) direct combination with plant constituents,

(2) hydroxylation of the compounds,

(3) cleavage of the ester linkages of the compounds.

CHAPTER 2

Selective Use of Phenmedipham and EP-475 in Michigan for Weed Control in Sugar Beets

Abstract

Research was conducted to examine the possible use of phenmedipham (methyl *m*-hydroxycarbanilate *m*-methylcarbanilate) and EP-475 (ethyl *m*-hydroxycarbanilate carbanilate) for weed control in sugar beets (*Beta vulgaris* L.). Various treatments of phenmedipham, EP-475 and pyrazon (5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone) were applied to sugar beets postemergence with and without a preemergence treatment of pyrazon + TCA (trichloroacetic acid) at various locations in different years. Weed control was greater with preemergence plus postemergence combination than with pre- or postemergence alone. Crop injury resulting from combination treatments did not affect yields. Double postemergence applications did not adversely affect the crop or recoverable sugar content compared to single postemergence treatments. EP-475 was necessary for redroot pigweed (*Amaranthus retroflexus* L.) control.

Introduction

Effective herbicidal weed control in Michigan sugar beets frequently involves the combined use of preemergence and postemergence applications. The trend toward minimum labor used in sugar beet fields necessitates total weed control.

The preemergence herbicide treatment often used on heavier soils in Michigan is a pyrazon plus TCA at 4.5 kg + 6.7 kg/ha (4 lb + 6 lb/A). In a favorable environment, good control of many broadleaf and grass weeds is obtained. However, frequently an application of a post-emergence herbicide such as phenmedipham is needed to control many of the escaped broadleaf weeds or some grasses (1, 2, 3, 4). Redroot pigweed is an annual broadleaf weed that phenmedipham will not control. EP-475, an analog of phenmedipham, is effective on pigweed (8).

Phenmedipham and EP-475 have phytotoxic activity only when applied as a foliage treatment. Upon contact with soil, the chemicals are no longer active[1] (5, 9).

Phenmedipham and EP-475 may cause foliar injury to sugar beets under adverse environmental conditions or when used in combinations with preemergence herbicides. However, crop stand, final yield, and sugar content are usually not affected[2] (2, 4, 8). Addition of nonphytotoxic oils or concentrates will increase herbicidal activity of these compounds on many weed species (6, 8).

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[1]Kossman, K. 1969. Phenmedipham residues in plant and soil. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Scherling AG. 1969. p. 16-20.

[2]Dawson, J. H. 1971. Effects of herbicides on sugar beets. Weed Sci. Soc. Amer. Abstr. No. 3.

The objective of this research was to examine the possible use of phenmedipham and EP-475 for weed control in sugar beets, by evaluating the efficacy, effect on yield, and effect on recoverable sugar content of the sugar beet root by these compounds.

Materials and Methods

To evaluate the efficacy of these two compounds, research plots were maintained on farmers' sugar beet fields at different locations in Michigan. Plot size was 3 or 4 70-cm (28 in) wide rows by 13.5 m (45 feet) long arranged in a randomized complete block design with three replications. All applications were broadcast by a tractor-mounted sprayer in 215 L/ha (23 gpa) of water (7).

In 1971, preemergence and postemergence treatments alone and in combination were applied to sugar beets on a sandy loam soil with 4 percent organic matter in Lenawee County, Michigan. Preemergence treatments were applied on April 12 and postemergence treatments were applied on May 11, when the sugar beet was in the 2-leaf stage. Rainfall within 1 week after preemergence treatment was 1.3 cm (0.5 in) and the total rainfall received for a 3-week period after application was 1.7 cm (0.7 in), the latter being 70 percent below the seasonal mean.

At a second location in 1971, similar applications were made on a clay loam soil with 12 percent organic matter in Saginaw County, Michigan. Preemergence applications were made on April 23 and postemergence applications were made on June 2, when sugar beets had full leaves. Rainfall within 1 week after preemergence application was 0.2 cm (0.1 in) and within 4 weeks was 1.4 cm (0.6 in), the latter being 56 percent below the seasonal mean.

In 1972, the soil used in Lenawee County was a sandy loam with 2 percent organic matter. Preemergence treatments were applied on April 28 and the postemergence treatments applied on May 17, when the first part of sugar beet leaves were one-half expanded. Rainfall within 1 week after preemergence application was 3.5 cm (1.4 in), and within 4 weeks after application was 7.3 cm (2.9 in), which approximates the seasonal mean.

In 1972, a second location was used in Bay County, Michigan with a sandy clay loam soil with 33 percent organic matter. Preemergence treatments were applied on May 9 and postemergence treatments applied on June 5, when the sugar beet was in the two-leaf stage, and again on June 14 on selected plots. Rainfall within 7 days after preemergence application was 0.5 cm (0.2 in) and within 4 weeks was 2.1 cm (0.8 in), the latter being 52 percent below seasonal mean.

Visual ratings of herbicidal effectiveness were usually obtained 1 to 2 weeks after postemergence application. Ratings on crop injury represent initial crop injury. Yields were taken in 1971 at Lenawee County by harvesting the center two rows of the 4-row plots. In 1972, juice from samples of sugar beet roots were taken from plots in Bay County and analyzed for percent recoverable sugar at Michigan Sugar Company, Saginaw, Michigan. Yields and sugar contents were analyzed for significant differences.

Results and Discussion

The weed control due to postemergence and preemergence plus postemergence herbicide combinations are shown in Tables 1, 2, 3, 4, and 5. Crop injury due to postemergence applications only was low except for the combinations shown in Table 1. Especially notable was the lack of sugar beet injury due to two postemergence applications (Tables 4, 5). Stand counts were made in 1972 but no significant differences due to treatments were observed.[3]

In general, weed control was greater with a combination of treatments. When more than 10 cm (0.4 in) of rain fell the first week after preemergence application, the best weed control was obtained (Tables 1 and 2). Due to drier soil conditions at two locations, the preemergence application resulted in poor weed control (Tables 3 and 4). However, when the postemergence treatments were applied, a substantial increase in weed control was obtained over

[3]Meggitt, W. F. and L. W. Hendrick, unpublished data, 1972.

Table 1. Visual weed control ratings of postemergence and preemergence plus postemergence herbicidal combinations in sugar beets in Lenawee County, Michigan, 1971.[a]

Treatment	Rate	Postemergence only			Preemergence + postemergence combination		
		Crop injury	Redroot pigweed	Lambs-quarters[b]	Crop injury	Redroot pigweed	Lambs-quarters
	(kg/ha)						
Pyrazon + phen-medipham	2.24+1.12	0.0	8.0	7.3	3.7	10.0	10.0
Phenmedipham	1.12	0.0	9.0	8.7	5.0	10.0	10.0
Phenmedipham	1.68	0.7	9.3	8.3	4.3	10.0	10.0
SN503	1.12	0.0	9.3	9.3	4.0	10.0	10.0
SN503	1.68	0.3	10.0	9.3	4.7	10.0	10.0
EP-475	1.12	0.7	9.7	9.0	5.0	10.0	10.0
EP-475	1.68	1.7	10.0	9.0	6.0	10.0	10.0
No postemergence application	-	0.0	0.0	0.0	0.7	9.7	7.7

[a]0=no injury or no control, 10=complete control or kill.

The preemergence herbicide application consisted of pyrazon + TCA at 2.24 + 6.72 kg/ha.

[b]Lambsquarters (_Chenopodium album_ L.)

Table 2. Visual weed control rating of postemergence and preemergence plus postemergence herbicidal combinations in sugar beets in Lenawee County, Michigan, 1972.[a]

		Postemergence only			Preemergence + postemergence combination		
Treatment	Rate	Crop injury	Redroot pigweed	Broad-leaves[b]	Crop injury	Redroot pigweed	Broad-leaves
	(kg/ha)						
Pyrazon + phenmedipham + oil	2.24+0.84+3.8 L	0.3	2.3	4.0	2.0	10.0	10.0
Pyrazon + EP-475 + oil	2.24+0.56+3.8 L	0.3	1.3	1.0	1.0	10.0	10.0
Phenmedipham + oil	1.12+3.8 L	0.3	1.3	2.0	1.7	9.5	10.0
Phenmedipham	1.68	0.0	1.0	1.0	0.7	9.5	10.0
SN503	1.12	0.0	1.7	4.0	1.3	10.0	10.0
SN503	1.68	0.7	1.7	8.0	0.7	9.8	10.0
SN503 + oil	1.12+3.8 L	0.7	1.7	1.3	1.7	10.0	10.0
EP-475 + oil	0.84+3.8 L	0.7	1.0	0.5	1.0	10.0	10.0
EP-475	1.68	0.0	-	-	1.3	10.0	10.0
No postemergence application	-	0.0	0.0	0.0	0.0	8.5	9.8

[a]0=no injury or no control, 10=complete control or kill.

[b]Broadleaves consisted of predominantly lambsquarters (*Chenopodium album* L.) with some purslane (*Portulaca oleracea* L.), and ragweed (*Ambrosia artemisiifolia* L.).

The preemergence herbicide application consisted of pyrazon + TCA at 3.36 + 6.72 kg/ha.

Table 3. Visual weed control ratings of postemergence and preemergence plus postemergence herbicidal combinations in sugar beets in Saginaw County, Michigan, 1971.[a]

Treatment	Rate	Postemergence only			Preemergence + postemergence combination		
		Crop injury	Redroot pigweed	Broadleaves[b]	Crop injury	Redroot pigweed	Broadleaves
	(kg/ha)						
Pyrazon + phenmedipham	2.24+1.12	0.0	3.7	7.0	0.0	4.0	9.3
Phenmedipham	1.12	0.0	0.7	6.3	0.0	0.0	10.0
Phenmedipham	1.68	0.7	0.0	7.3	1.0	3.3	10.0
SN503	1.12	0.3	7.3	9.0	1.0	8.0	9.7
SN503	1.68	0.3	7.7	6.0	2.3	10.0	9.7
EP-475	1.12	0.7	9.7	2.0	0.3	8.3	8.7
EP-475	1.68	1.7	9.3	3.7	0.7	9.7	8.7
No postemergence application	-	0.0	0.0	0.0	0.0	0.0	0.0

[a]0=no injury or no control, 10=complete control or kill.

[b]Broadleaves consisted of predominantly lambsquarters (Chenopodium album L.), with some wild buckwheat (Polygonum convolvulus L.).

The preemergence herbicide application consisted of pyrazon + TCA at 4.48 + 6.72 kg/ha.

Table 4. Visual weed control ratings of postemergence and preemergence plus postemergence herbicidal combinations in sugar beets in Bay County, Michigan, 1972.[a]

Treatment	Rate	Postemergence only			Preemergence + postemergence combination		
		Crop injury	Redroot pigweed	Broadleaves[b]	Crop injury	Redroot pigweed	Broadleaves
	(kg/ha)						
Pyrazon + phenmedipham + oil	2.24+1.12 +3.8 L	1.0	5.2	9.8	1.3	7.3	10.0
Pyrazon + EP-475 + oil	2.24+0.56 +3.8 L	0.3	8.8	7.8	1.7	9.5	8.0
Phenmedipham + oil	1.12+3.8 L		1.0	9.2	0	1.3	10.0
Phenmedipham	1.68	1.0	3.0	10.0	2.0	5.7	10.0
SN503	1.12	1.0	6.3	8.0	1.0	9.5	10.0
SN503	1.68	1.7	9.3	9.5	1.3	9.8	10.0
SN503 + oil	1.12+3.8 L	1.3	7.3	9.3	1.0	9.0	9.8
EP-475 + oil	0.84+3.8 L	1.0	9.5	7.8	0.3	9.8	9.2
EP-475	1.68	0.3	9.7	9.7	2.0	10.0	9.7
No postemergence application	-	0.0	0.0	0.0	0.0	0.0	3.3

[a]0=no injury or no control, 10=complete control or kill.

[b]Broadleaves consisted of lambsquarters (Chenopodium album L.) and also wild buckwheat (Polygonun convolvulus L.), common ragweed (Ambrosia artemisiifolia L.) and Pennsylvania smartweed (Polygonum pensylvanicum L.).

The preemergence herbicide application consisted of pyrazon + TCA at 4.48 + 6.72 kg/ha.

Table 5. Visual weed control ratings of double postemergence and preemergence plus double postemergence combinations in sugar beets in Bay County, Michigan, 1972.[a]

Treatment	Rate	Double postemergence treatments only: Crop injury	Redroot pigweed	Lambs-quarters[b]	Preemergence + double postemergence treatments: Crop injury	Redroot pigweed	Lambs-quarters
	(kg/ha)						
Pyrazon + phen-medipham + oil	2.24+1.12+ 3.8 L 2.24+0.56+ 3.8 L	1.3	9.1	9.7	1.7	9.5	10.0
Pyrazon + EP-475 + oil	2(2.24+0.56 +3.8 L)	1.3	9.3	8.0	1.3	10.0	8.5
Phenmedipham + oil	1.12+3.8 L 0.56+3.8 L	1.0	1.7	9.7	1.7	4.0	10.0
Phenmedipham	1.68 0.84	1.0	4.7	10.0	2.3	4.7	10.0
SN503	2(0.84)	0.3	8.8	9.7	1.0	9.2	9.8
SN503 + oil	1.12+3.8 L 0.56+3.8 L	3.3	9.5	10.0	2.7	10.0	9.8
EP-475 + oil	2(3.35+ 3.8 L)	1.7	9.7	8.3	2.0	9.7	10.0
EP-475	1.68 0.84	0.3	9.8	9.3	1.3	10.0	10.0
No postemergence application	-	0.0	0.0	0.0	0.0	0.0	3.3

[a]0=no injury or no control, 10=complete control or kill.

[b]Lambsquarters (Chenopodium album L.)

The preemergence herbicide application consisted of pyrazon + TCA at 4.48 + 6.72 kg/ha.

postemergence applications alone. This indicates that, even though no visual toxicity to susceptible weeds was seen, the preemergence treatment affected these plants sufficiently to allow much greater phytotoxicity by the postemergence herbicides.

Phenmedipham controled redroot pigweed as well as many other broadleaf weeds in the cotyledonary and prior to the full two-leaf stage (Table 1). Larger redroot pigweed plants were not controlled by phenmedipham, but activity was increased when pyrazon was added to the postemergence mixture (Tables 2, 3, 4, and 5) (2, 8). EP-475 controlled pigweed effectively at 0.8 kg/ha (3/4 lb/A) a.i. with a nonphytotoxic oil or 1.12 kg to 1.68 kg/ha (1 lb to 1.5 lb/A) or greater without an oil.

Mixtures of phenmedipham and EP-475 can also be used. Lower rates of SN503[4], a 1:1 mixture of phenmedipham and EP-475, controlled many broadleaves, but higher rates were necessary to control pigweed.

If the weeds had not emerged, a postemergence application of a herbicide alone that does not exhibit soil activity after application was not effective (Table 2).

A high amount of crop injury was observed with herbicide combinations at Lenawee County in 1971 (Table 1). With this amount of initial foliar inhibition, it seemed

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[4]Designation by Nor-Am Agricultural Chemicals, Woodstock, Illinois.

possible that yields would be affected. However, an analysis of variance performed on yields of various plots showed there were no significant differences among the mean yields (Table 6).

Also of concern was the effect of herbicides on the recoverable sugar content in the root. As shown in Table 7, an analysis of variance indicated no significant differences due to single or double applications of post-emergence treatments combined with the preemergence treatment.

It was advantageous to use combinations of preemergence and postemergence herbicides to obtain the greatest amount of weed control in sugar beets and not adversely affect the yields of roots even though considerable foliar injury results. Split applications of postemergence herbicides gave excellent weed control. Sugar beet injury and recoverable sugar content was not adversely affected.

Table 6. Yields of sugar beets in Lenawee County, Michigan, 1971.[a]

Postemergence treatment	Rate	Mean Yield
	(kg/ha)	(1000 kg/ha)
Pyrazon + phenmedipham	2.24+1.12	66.8
Pyrazon + phenmedipham + oil	2.24+0.84+3.8 L	60.5
Phenmedipham	1.12	59.1
Phenmedipham	1.68	66.3
SN503	1.12	70.1
SN503	1.68	59.6
EP-475	1.12	73.0
EP-475	1.68	70.3
No postemergence treatment	-	67.6
Check	-	67.4

[a]All above treatments, except the check, received a preemergence application of pyrazon + TCA at 3.36 + 6.72 kg/ha. The above means were not significantly different by an AOV at the 5 percent level.

Table 7. Kilograms recoverable white sugar per 1000 kg of sugar beet roots in Bay County, Michigan, 1972.

Postemergence treatment	Rate	Weight
	(kg/ha)	(kg)
Receiving two postemergence treatments		
No postemergence application		150.5
Pyrazon + phenmedipham	2.24+1.12+3.8 L	
+ oil	2.24+0.56+1.12	149.6
Pyrazon + EP-475 + oil	2(2.24+0.56+1.12)	139.4
Phenmedipham + oil	1.12+1.12	
	0.56+1.12	146.5
EP-475 + oil	2(0.84+1.12)	153.4
Phenmedipham	1.68	
	0.84	138.3
EP-475	1.68	
	0.84	148.8
EP-475	0.84	
	0.84	143.1
SN503	0.84	
	0.84	148.9
SN503	1.68	147.4
SN503	1.68	
	0.84	151.8
SN503 + oil	1.12+1.12	
	0.56+1.12	140.0
SN503 + oil	1.68+1.12	
	0.84+1.12	155.8
Receiving one postemergence treatment		
Pyrazon + EP-475 + oil	2.24+0.56+1.12	136.3
EP-475 + oil	0.84+1.12	157.0
EP-475 + oil	1.12+1.12	150.0
EP-475	1.68	150.7
SN503	1.12	145.6
SN503	1.68	146.2
SN503 + oil	1.12+1.12	140.2
SN503 + oil	1.68+1.12	149.5
Check	-	154.5

[a]All above treatments, except the check, received a preemergence application of pyrazon + TCA at 4.48 + 6.72 kg/ha. The above means were not significantly different by an AOV at the 5 percent level.

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CHAPTER 3

Basis for Selectivity of Phenmedipham and EP-475 on Wild Mustard, Redroot Pigweed, and Sugar Beet

Abstract

Studies were initiated to determine the basis for selectivity of phenmedipham (methyl-m-hydroxycarbanilate m-methyl carbanilate) and EP-475 (ethyl m-hydroxycarbanilate carbanilate) on wild mustard [Brassica kaber (DC.) L.C. Wheeler "pinnatifida" (Stokes) L.C. Wheeler], redroot pigweed (Amaranthus retroflexus L.) and sugar beet (Beta vulgaris L.) by evaluating spray retention, absorption, translocation and metabolism. Total photosynthesis in wild mustard was severely inhibited in less than 5 hr after foliar application of either herbicide and did not recover. Total photosynthesis in sugar beet was slightly inhibited but recovered during the observation period. Photosynthesis in redroot pigweed recovered from a treatment of phenmedipham but did not recover when treated with EP-475. There were no differences observed in spray retention on the leaf surfaces between the herbicides or among the species.

Differences in foliar absorption of the herbicide also did not indicate why the herbicides differed in their activity on redroot pigweed. Within 5 hr after herbicide application, redroot pigweed had translocated more EP-475 than phenmedipham from the site of absorption. Within 5 hr after application, sugar beet had metabolized a large

amount of phenmedipham and EP-475, but wild mustard had not. In this time redroot pigweed had metabolized a large amount of phenmedipham but little EP-475. The key factor explaining selectivity appeared to be at the initial detoxication reaction of the parent compound as similar amounts of various metabolites were found in all three species treated with each herbicide.

Introduction

The herbicides phenmedipham and EP-475 (Figure 1) are both applied postemergence to sugar beets for control of many broadleaf and some grass weeds (1, 4, 6, 12). However, only EP-475, an analog of phenmedipham, controls redroot pigweed once it reaches the two leaf or older stage.

Phenmedipham and EP-475 both contain the peptide bond structure common to many photosynthetic inhibitors (5, 11). Phenmedipham has been shown to inhibit CO_2 assimilation by isolated chloroplasts from both susceptible and resistant plants (1). However, with intact plants, the susceptible plants did not recover from the initial inhibition of CO_2 assimilation as did resistant plants[1] (1, 9). Research using isolated chloroplasts did not explain the basis for

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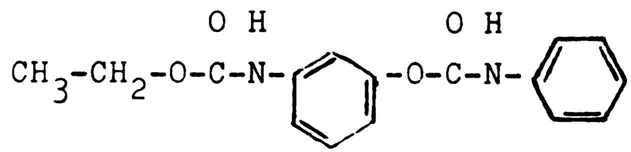
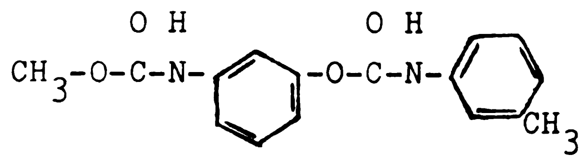
[1]Willenbrink, J. 1969. The action of phenmedipham on the CO_2 fixation during photosynthesis in young sugar beet leaves. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Schering AG. 1969, 7-9.



EP-475



Phenmedipham

Figure 1. Molecular structure of EP-475 (top) and phenmedipham (bottom).

selectivity of these herbicides on certain plant species (3). Kassebeer[2] (7) found that the greater part of the phenmedipham applied had been absorbed into plants 4 hr after treatment. Only acropetal movement from the site of application was observed, and this was evident within 8 hr after treatment. In wild mustard, a rapid distribution throughout the plant was observed. Bischof (2) found that sugar beet and white mustard (Brassica hirta Moench) absorbed similar amounts of foliar-applied phenmedipham.

Sugar beet, and redroot pigweed to a lesser degree, rapidly metabolized phenmedipham to less toxic compounds (7). EP-475 was also metabolized in sugar beet in time (8). Bischof (2) found that neither uptake nor rate of degradation alone explained the selectivity of phenmedipham but rather a combination of the two factors producing a high or low amount of active ingredient in the plants.

The purpose of this research was to find the basis for selectivity of phenmedipham and EP-475 in sugar beet, redroot pigweed, and wild mustard by evaluating spray retention, absorption, translocation and metabolism. The latter specie is susceptible to both compounds, whereas redroot pigweed is susceptible to only EP-475.

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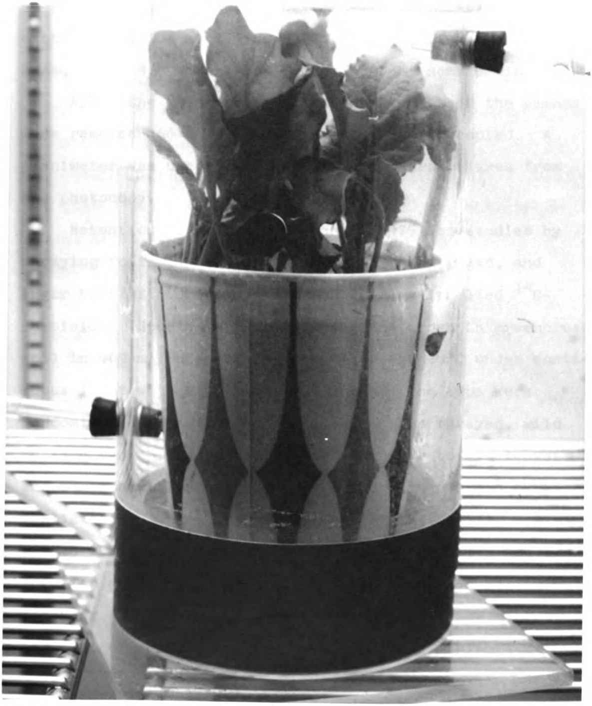
[2]Kassebeer, H. 1969. The absorption, translocation and decomposition of phenmedipham marked ^{14}C and ^{3}H in young beets (Beta vulgaris) and weeds. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Scherling AG. 1969, p. 15.

Materials and Methods

Four pigweed plants, or three to four mustard plants, or four sugar beet plants per 909-ml cup were grown in greenhouse soil until the plants had eight, six, and four leaves, respectively. The plants were grown in the growth chamber at 25 C in light of 24,000 to 27,000 lux with 16 hr of day-length. Photosynthesis and respiration measurements were made by placing the cup of plants one at a time in a sealed clear plastic test chamber (Figure 2) which in turn was located in a growth chamber similar to that in which the plants were grown, and attaching the test chamber to a Beckman Model IR215 CO_2 infrared gas analyzer by means of Tygon[R] tubing. Air from a compressed air source was passed through the chamber at a rate of 500 cc per min.

Results were recorded on the Beckman Ten-Inch Laboratory Potentiometric Recorder. The analytical system was adjusted to zero on the recorder with nitrogen and to 50% deflection with compressed air without plants in the chamber. The plants were then placed in the plastic test chamber, the lights were turned on which permitted the plants to photosynthesize, thereby lowering the CO_2 content of the effluent gas, until the recorder gave a straight horizontal line response which indicated that equilibrium had been obtained. The lights were turned off allowing the plants to respire. This increased the CO_2 content of the effluent gas until again the recorder gave a straight horizontal line response.

Figure 2. Sealed plastic chamber used for photosynthesis and respiration studies.



One unit of change on the recorder paper was equivalent to 6.46 mg of CO_2 per min (Appendix A). Measurement on these plants was taken prior to treatment and 4, 30, 45, and 85 hr after treatment. Plants were sprayed topically in a "movable boom" spray chamber with formulations of either herbicide at 1.7 kg/ha in 280 L/ha of water and 2.1 kg/sq cm at 4.8 km/hr with TeeJet^R 8004E[3] nozzle tips.

After the 85 hr observation, the leaves of the plants were removed and stapled onto paper and photocopied. A planimeter was used to obtain the leaf surface area from the photocopy.

Retention of phenmedipham and EP-475 was studied by spraying foliage of wild mustard, redroot pigweed, and sugar beet with uniformly side-chain ring labelled ^{14}C-herbicide. Species were germinated and grown in greenhouse soil in 909-ml cups in growth chambers at 25 C under continuous light with 24,000 to 27,000 lux. The cups were randomized daily within the chamber. When sprayed, wild mustard had three leaves, pigweed had five to six leaves and sugar beet has two leaves. All treatments had three replications. The ^{14}C-phenmedipham had a specific activity of 5.5 mCi/millimole and was 96% pure; ^{14}C-EP-475 had a specific activity of 1.95 mCi/millimole and was 98% pure. Spraying was done as described above at the rate of 1.1 kg/ha ai of cold formulated herbicide spiked with 1 uCi

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[3]Spraying Systems Company, Wheaton, Illinois.

(or 2 uCi in second experiment) of radioactive herbicide per 100 ml of solution.

After application, the plants were allowed to dry and then the leaves were removed and rinsed in 45 to 50 ml of acetone for 15 sec to elute the herbicide from the leaf. Leaf areas were obtained in the same manner as described above. The amount of radioactivity obtained was determined by Packard Tri-Carb Liquid Scintillation Spectrometer. The scintillation solution used consisted of 0.1 g of 1,4-bis [2-(4-methyl-5-phenyloxazolyl)]-benzene (dimethyl-POPOP), 5.0 g of 2,5-diphenyloxazole (PPO), 50.1 g of naphthalene, 380 ml of toluene, 380 ml of 1,4-dioxane, and 240 ml of absolute ethanol.

Translocation of these two herbicides in the three species was studied by using ^{14}C-herbicides foliarly applied into lanolin rings. The species were germinated in vermiculite and transplanted into #7 white quartz sand in 909-ml cups at the cotyledon stage. These cups were watered subterraneally with full-strength Hoagland's solution. The growth chamber environmental conditions were 25 C under continuous light at 24,000 to 27,000 lux. The cups were randomized daily within the chamber.

When treated, wild mustard had four to six leaves, pigweed had six to eight leaves and sugar beet had two leaves. A 5 to 6-mm diameter lanolin ring was centered at the basipetal end of the second oldest leaf on each of two replications. A 5-ul drop containing 0.1 uC of

the radioactively labelled herbicide was placed into the lanolin ring, allowed to dry, and the plants returned to the growth chamber. The radioactive herbicides were dissolved in EP-475 solvent system[4]: water (1:20,v/v)(10). After each time period of 1, 3, or 6 days after application, the lanolin ring was removed, roots washed free of sand and the intact plants freeze-dried. These plants were then mounted onto white absorbent paper and radioautographed using Kodak No-screen X-ray film.

To determine the type of distribution of the herbicides within the plants, a root uptake study was done. The species were germinated in vermiculite and transplanted into #7 white quartz sand at the cotyledon stage in the growth chamber. The plants were watered subterraneally with full-strength Hoagland's solution. The environmental conditions used throughout were 25 C under continuous light at 13,000 to 16,000 lux. When wild mustard was in the four leaf stage, pigweed in the six leaf stage, and sugar beet in the two to four leaf stage, the plants were washed free of sand and transferred into 20 x 80 mm vials containing 10 ml of 1/2-strength Hoagland's solution. The plants were supported by a foam rubber stopper. After equilibration for 24 hr in the growth chamber, this solution was replaced with 10 ml of 1/2-strength Hoagland's spiked with

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[4]Supplied by Nor-Am Agricultural Chemicals, Woodstock, Illinois.

1 uC of either radioactively labelled herbicide per 200 ml of solution and replaced in the growth chamber. Twelve hr later the plants were taken out of the vials, roots washed twice in distilled water, mounted onto white absorbent paper, and radioautographed.

Studies on absorption and metabolism of these compounds by the three species mentioned were conducted in the growth chamber. The three species were germinated and grown in greenhouse soil in 909-ml cups in the growth chamber at 25 C in continuous light of 13,000 to 16,000 lux. At the same stage as above, 0.1 uC of each labelled herbicide was applied to the second oldest leaf of each species into a 7 to 8-mm ink-marked ring centered at the basipetal portion of the leaf. Care was taken not to allow spreading of the applied droplet outside of the ink ring. There were three replications per treatment.

After 5 and 24 hr, the treated leaf was removed and three types of extractions made. The area of the treated leaf that was acropetal to the point of application was cut off and designated as "tip". The area within the inked ring was cut out of the leaf, rinsed in acidified acetone for 15 sec, and set aside, labelled as "spot". Acidified acetone was prepared by adding 1 ml of 1 N HCl into 1 L of acetone. The acetone rinse was collected into 56-ml bottles, which should contain all ^{14}C-labelled herbicide that was not absorbed into the cellular tissue. The spot and tip tissue each were homogenized in a Sorvall

Omni-Mixer for 2 min in 15 ml of acidified acetone. The homogenate was vacuum filtered through #5 filter paper into 57-gm bottles and the collected residue weighted and combusted by the Schoniger combustion method (13). The three acetone-soluble leaf fractions collected from one leaf were treated similarly in the rest of the procedure. The bottles were flash-evaporated, chilled, and 200 ml of cold methanol added. Of this, 50 ul was spotted onto 250 nm thick (20 by 20 cm) silica gel GF-254 (Brinkmann Instruments) thin layer chromatography (TLC) plates developed in chloroform: isopropylether (9:1,v/v)(hereafter called solvent system I) and radioautographed. Any position, except at that Rf equivalent to the parent material, on the plate that exposed the X-ray was scraped from the plate, eluted with methanol, filtered into 28-ml bottles, dried by flash evaporation and chilled. Of the 200 ul of methanol added to the bottle, 150 ul was spotted on similar TLC plates as above and developed in a more polar solvent system, butanol:water:acetic acid (12:5:3, v/v/v) (hereafter called solvent system II) and then radioautographed. The positions that were equivalent in Rf of the parent material were scraped in liquid scintillation vials and radioassayed.

The TLC plates that were developed in the more polar solvent system were delineated into four areas according to the greater prominance of exposed areas to the X-ray film, scraped into liquid scintillation vials, and radioassayed.

Results and Discussion

Phenmedipham and EP-475 application reduced the CO_2 uptake of all three species within 4 hr after treatment (Table 1). Total photosynthesis in wild mustard was greatly reduced by both chemicals and did not recover within the 85 hr observation period. Sugar beet was not as adversely affected and recovered with time. Total photosynthesis of pigweed was greatly reduced but showed recovery in the plants sprayed with phenmedipham but not in plants sprayed with EP-475.

Arndt and Kotter (1) and Willenbrink[5] also found that phenmedipham inhibited photosynthesis in sugar beet, redroot pigweed, and wild mustard but photosynthesis in sugar beet and redroot pigweed recovered with time.

Dark respiration of phenmedipham-treated wild mustard, redroot pigweed, and sugar beet and EP-475-treated sugar beet was not reduced (Table 2). However, dark respiration of EP-475-treated wild mustard and redroot pigweed was reduced and did not increase with time. Arndt and Kotter (1) found that dark respiration of wild mustard was permantly inhibited by phenmedipham but sugar beet recovered

[5]Willenbrink, J. 1969. The action of phenmedipham on the CO_2 fixation during photosynthesis in young sugar beet leaves. Scientific and practical experience in using phenmedipham for weed control in beets, Berlin, January 1969. Abstr. (of conf. sponsored by) Schering AG. 1969, p. 7-9.

Table 1. Mean percent of initial total photosynthesis of three species due to herbicide treatment.

Herbicide Species		Hours After Treatment[a] 4	30	45	85
Phenmedipham					
Wild mustard	*[b]	17.2 abc	12.7 abc	16.5 abc	23.3 cd
Redroot pigweed	*	33.3 d	49.0 e	52.9 e	71.6 f
Sugar beet	*	83.9 fg	100.9 hi	99.6 hi	106.9 i
EP-475					
Wild mustard	*	17.4 abc	6.9 a	9.2 ab	14.2 abc
Redroot pigweed	*	15.7 abc	4.4 a	5.1 a	22.5 bcd
Sugar beet	*	79.8 fg	91.5 gh	83.8 fg	88.9 gh

[a]Means followed by like 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 after herbicide treatment using students "T" test.

Table 2. Mean percent of initial dark respiration of three species due to herbicide treatment.

Herbicide Species		Hours After Treatment[a] 4	30	45	85
Phenmedipham					
Wild mustard		69.6 a-e	61.1 a-d	57.5 abc	53.9 abc
Redroot pigweed		86.6 d-i	91.8 e-i	97.1 f-i	111.6 hi
Sugar beet		77.3 c-h	94.7 g-i	96.8 hi	106.1 i
EP-475					
Wild mustard	*[b]	71.7 b-f	47.0 ab	55.5 abc	60.3 a-d
Redroot pigweed	*	74.0 b-g	59.0 a-d	41.8 a	55.2 abc
Sugar beet		87.8 e-i	90.4 e-i	104.0 i	92.8 e-i

[a]Means followed by like letters are not significantly different at the 5% level using Duncan's Multiple Range Test.

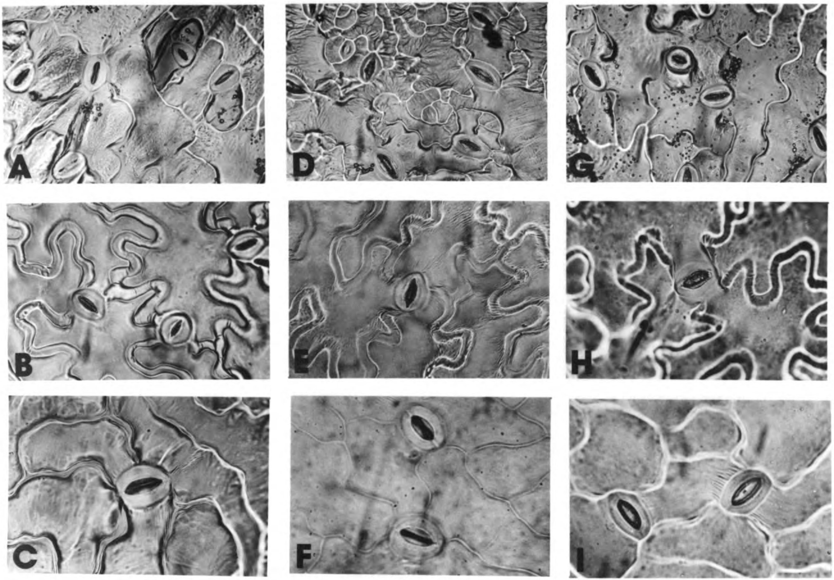
[b]Asterisks indicate significance between the amount of photosynthesis prior to and 4 hr after herbicide treatment using Student's "T" test.

in time. Neither the effect of these herbicides on photosynthesis nor respiration explained the selectivity. A decrease in photosynthesis can result from the closing of the stomates. Using clear fingernail polish, impressions of the lower surfaces of treated and untreated plants were made and photographed (Figure 3). Observations over a large area (1 sq cm) of the impressions indicated little or no difference in stomate aperture.

There were no significant differences in the amount of herbicide retained on the upper surfaces of leaves between the two herbicides or among the three species (Table 3). So this factor did not contribute to selectivity. At 5 hr after treatment, there were no differences in the amount of herbicide absorbed between the herbicides or among the species (Table 4). At 24 hr after treatment, more phenmedipham was absorbed by these plants, except for pigweed which absorbed both chemicals in similar amounts. Bischof (2) also found that sugar beet and white mustard absorbed phenmedipham in similar amounts. The absorption of phenmedipham by redroot pigweed and sugar beet and of EP-475 by wild mustard and sugar beet had increased significantly between 5 and 24 hr after application. Thus the plant species continued to absorb herbicide during the time observed.

Once the herbicide has been absorbed, it may translocate to other plant parts. Phenmedipham and EP-475 were not translocated basipetally (Figure 4) from the point of application, even 6 days after application (Figure 5).

Figure 3. Microphotographs of lower leaf surfaces of EP-475 treated wild mustard (A), redroot pigweed (B), sugar beet (C); phenmedipham treated wild mustard (D), redroot pigweed (E), sugar beet (F); untreated wild mustard (G), redroot pigweed (H), sugar beet (I).


A
B
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I

Table 3. Amount of ^{14}C-herbicide retained on leaves of three species.[a]

Herbicide	Species	DPM/6.5 sq cm
Phenmedipham	Wild mustard	254
	Redroot pigweed	292
	Sugar beet	221
EP-475	Wild mustard	241
	Redroot pigweed	285
	Sugar beet	190

[a]The above means were not significantly different by an AOV at the 5% level.

Table 4. Absorption of ^{14}C-herbicides.

Herbicide	Species	Percent absorption[a]		
		5 hr		24 hr
Phenmedipham	Wild mustard	37.3 b		59.0 b
	Redroot pigweed	22.4 ab	*[b]	54.2 ab
	Sugar beet	31.6 ab	*	72.1 b
EP-475	Wild mustard	17.6 a	*	39.2 a
	Redroot pigweed	25.7 ab		39.1 a
	Sugar beet	22.5 ab	*	39.9 a

[a]Means within a column followed by like letters are not significantly different at the 5 percent level as determined by Duncan's Multiple Range Test.

[b]Asterisks between two means indicates significance using Student's "T" test.

Figure 4. Translocation of ^{14}C-phenmedipham (C) and ^{14}C-EP-475 (D) in sugar beet (left), redroot pigweed (center), and wild mustard (right) 1 day after foliar application. The treated plants are at top; the corresponding radioautographs are at the bottom.

Figure 5. Translocation of ^{14}C-phenmedipham (A) and ^{14}C-EP-475 (B) in sugar beet (left), redroot pigweed (center), and wild mustard (right) 6 days after foliar application. The treated plants are at top; the corresponding radioautographs are at the bottom.

A

B

However, there was acropetal translocation of both herbicides. Kassebeer[6] (7) observed that translocation of phenmedipham was solely acropetal. A difference in the distribution of the herbicides in the leaves can be seen when the herbicides were supplied to the roots (Figures 6 and 7). There was very little uptake of both herbicides in sugar beet, major restriction to the major veins in pigweed, and a more diffuse distribution in wild mustard.

Five hr after foliar application, wild mustard appeared to translocate more ^{14}C-herbicide acropetally from the point of application than the sugar beet but the difference was not statistically significant (Table 5). Redroot pigweed did translocate significantly more EP-475 than phenmedipham. Indications of higher amounts of EP-475 in redroot pigweed could not be detected on the radioautographs due to the veinal localization. There were no differences in the amount of translocation between herbicides or among species 24 hr after foliar application except that wild mustard continued to acropetally translocate EP-475 in significant amounts between 5 and 24 hr after application.

Of these amounts that did translocate acropetally within the treated leaf, differences in amount of parent compound metabolized can be seen 5 hr after foliar application (Table

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[6]Kassebeer, H. 1971. Aufnahmegeschwindigkeit, metabolismus and verlagerung von phenmedipham bei verschieden empfindlichen pflanzen. A. fur Pflanzenkrankheiten und Pflanzenschutz 18:158-174.

Figure 6. Distribution of root applied ^{14}C-phenmedipham in wild mustard (left), redroot pigweed (center), and sugar beet (right). The treated plants are on top; the corresponding radioautograph is at the bottom.

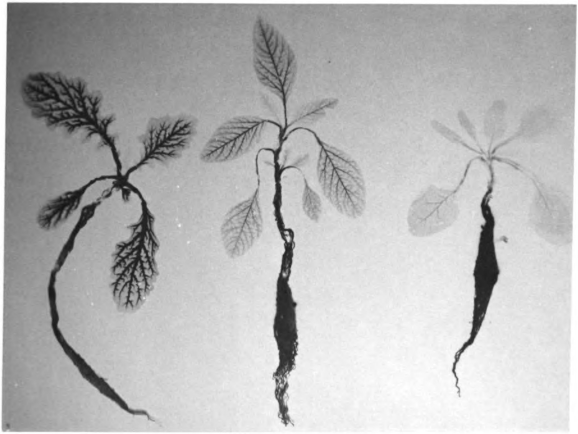

Figure 7. Distribution of root applied ^{14}C-EP-475 in wild mustard (left), redroot pigweed (center) and sugar beet (right). The treated plants are on top; the corresponding radioautograph is at the bottom.

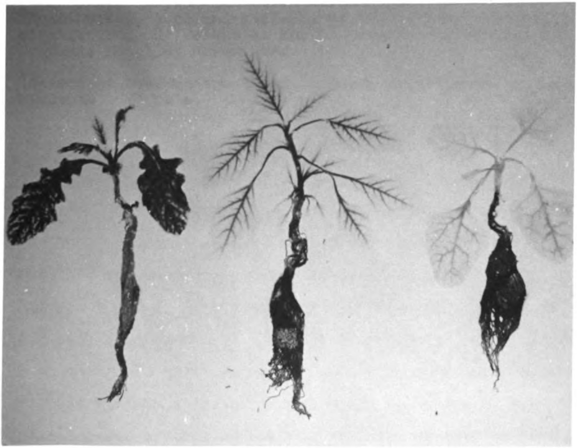

Table 5. Percent of ^{14}C from absorbed herbicide translocated acropetally in the same leaves of three species.

Herbicide	Species	Percent ^{14}C translocated[a] 5 hr		24 hr
Phenmedipham	Wild mustard	22.3 ab		32.4 ab
	Redroot pigweed	18.3 ab		28.1 ab
	Sugar beet	10.7 a		33.0 ab
EP-475	Wild mustard	29.5 b	*[b]	43.7 b
	Redroot pigweed	57.7 c		36.5 ab
	Sugar beet	21.2 ab		21.1 a

[a]Means within a column followed by like letters are not significantly different at the 5% level as determined by Duncan's Multiple Range Test.

[b]Asterisks between two means indicate significance using Students' "T" test.

6). Wild mustard did not metabolize as much phenmedipham or EP-475 but sugar beet did.

As the three species metabolized both herbicides, complexing with polar plant components was quick (Appendix B-1, B-2, and B-3). Very small amounts of radioactivity was found at Rf's other than those equal to the origin and parent compound when the extracts were chromatographed in solvent system I. Redroot pigweed metabolized large amounts of phenmedipham but metabolized only small amounts of EP-475. Metabolism of the herbicide continued between 5 and 24 hr after application. Redroot pigweed had metabolized such large amounts of phenmedipham 5 hr after application that the increase in the amount metabolized 19 hr later was not significantly different.

Kassebeer (7) had previously reported that sugar beet, and redroot pigweed to a lesser degree, rapidly metabolized phenmedipham. Knowles (8) found that foliarly applied EP-475 was metabolized with time by sugar beet.

Much of the above metabolized material remained at the origin of the TLC when chromatographed in the solvent system I. When rechromatographed in the solvent system II, ^{14}C-labelled compounds were distributed as illustrated in Figures 8 and 9. The ratio of the different metabolites for each species within either time period were similar for both herbicide treatments. So the ratio of various metabolites was not a factor in the basis for selectivity. If the ratio of various metabolites were similar and the

Table 6. Percent of translocated ^{14}C-herbicide metabolized after 5 and 24 hr.

Herbicide	Species	Percent metabolized[a] 5 hr		Percent metabolized[a] 24 hr
Phenmedipham	Wild mustard	30.16 b	*[b]	67.79 bc
	Redroot pigweed	70.32 c		74.43 cd
	Sugar beet	65.74 c	*	83.28 d
EP-475	Wild mustard	12.81 a	*	33.37 a
	Redroot pigweed	17.61 b	*	62.61 b
	Sugar beet	67.06 c	*	93.69 c

[a]Means within a column followed by like letters are not significantly different at the 5% level as determined by Duncan's Multiple Range Test.

[b]Asterisks between two means indicates significance using Students' "T" test.

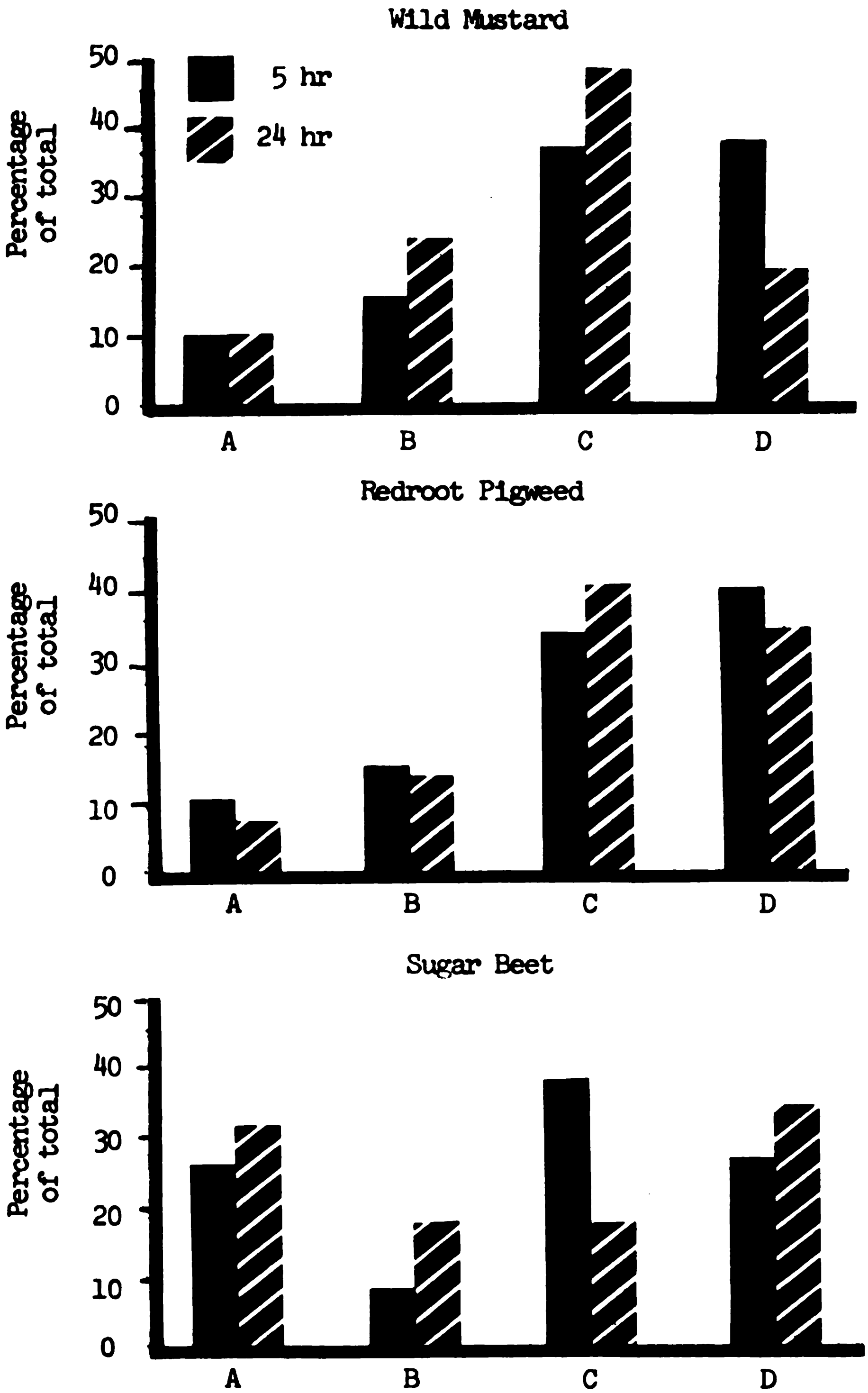

Figure 8. Chromatographic separation of phenmedipham metabolites using solvent system II. Rf for A=1.00-0.86; B=0.86-0.72; C=0.72-0.62; D=0.62-0.49.

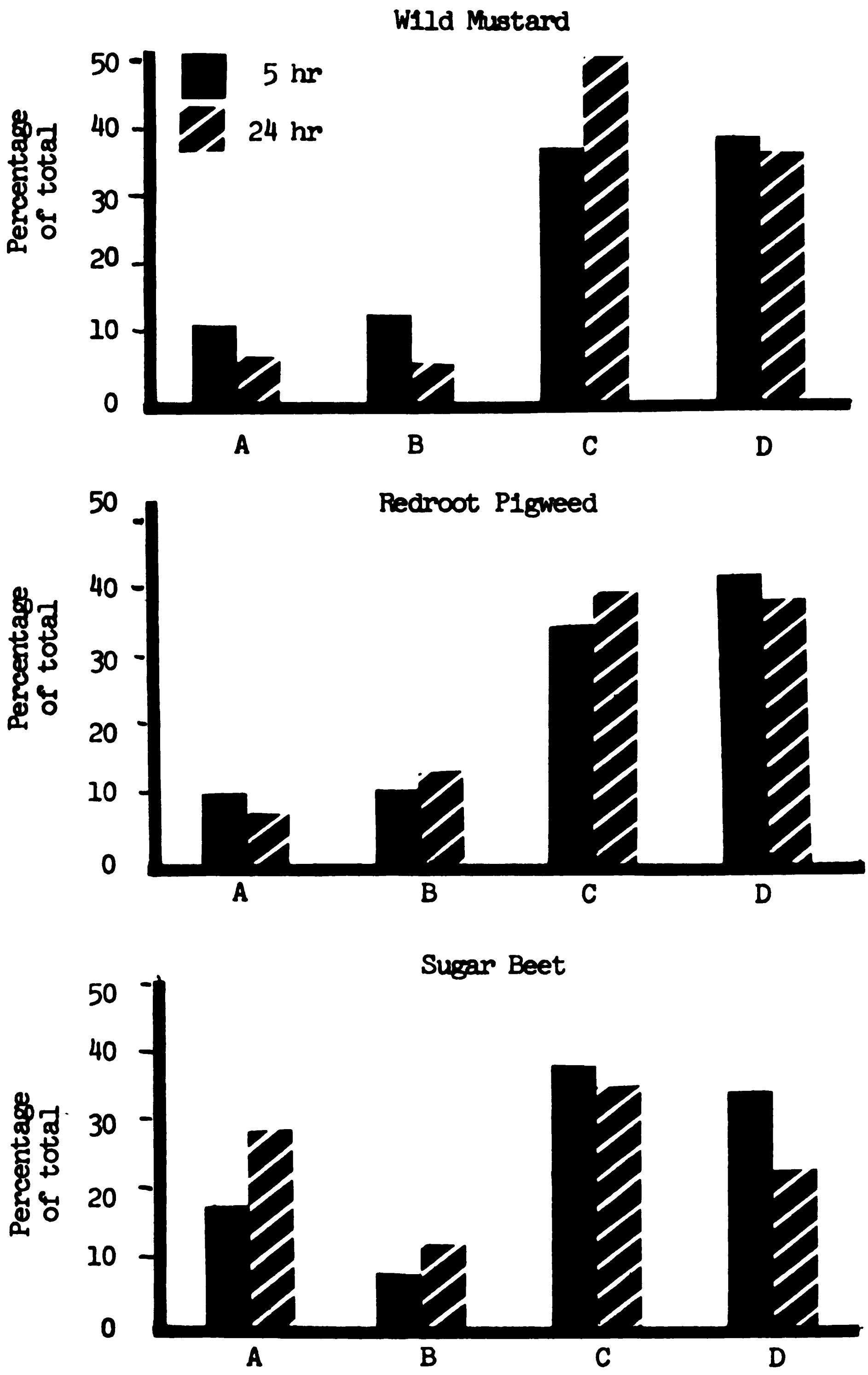

Figure 9. Chromatographic separation of EP-475 metabolites using solvent system II. Rf for A=1.00-0.86; B=0.86-0.72; C=0.72-0.62;D=0.62-0.49.

rates of possible changes between metabolites are similar, the site of differential metabolism must be in the first detoxication reaction involving the intact parent compound.

Selectivity of EP-475 and phenmedipham on redroot pigweed can be explained by increased translocation and decreased metabolism of EP-475. The metabolites acted in a polar manner, which could be conjugates of polar plant materials.

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CHAPTER 4

Summary and Conclusions

Studies in the field and greenhouse were conducted to evaluate the effect of phenmedipham and EP-475 on sugar beet, redroot pigweed, and wild mustard. Areas of investigation were efficacy in the crop and effects on yield and sugar content in the field. The effect on photosynthesis as related to spray retention, absorption, translocation, and metabolism were studied in the laboratory.

The results of these investigations are summarized as follows:

1. Combinations of preemergence and postemergence herbicides were usually needed for effective weed control.

2. Crop injury resulting from herbicide combinations did not affect final root yield.

3. No treatment affected the recoverable sugar content of the sugar beet root.

4. Phenmedipham used as a postemergence herbicide will control many common problem broadleaf weeds except redroot pigweed in sugar beet fields.

5. EP-475 alone or in combination with phenmedipham was necessary for redroot pigweed control.

6. At least 0.6 kg/ha (0.5 lb/A) of EP-475 was needed to obtain the needed redroot pigweed control.

7. Photosynthesis in sugar beet, redroot pigweed, and wild mustard was reduced within 5 hr after application of

either phenmedipham or EP-475 and the amount of reduction and recovery was directly related to the susceptibility of the specie.

8. There were no significant differences in spray retention on the leaf surface between both herbicides or among the species.

9. Differences in herbicide absorption did not help explain the difference in activity of the two herbicides on pigweed.

10. Herbicide translocation was solely acropetal.

11. Redroot pigweed translocated more EP-475 from the site of application than phenmedipham.

12. Significant amounts of herbicide continued to be translocated with time up to 24 hr after treatment.

13. Early distribution of both herbicides when root applied was very diffuse in wild mustard and primarily localized in the veins in redroot pigweed; however, sugar beet leaves appeared to contain even less herbicide in the primary veins than redroot pigweed.

14. Differences in the rate of metabolism of the parent compounds accounted for the difference in the susceptibility of the species, the susceptible species did not metabolize large amounts of herbicides quickly whereas the resistant species did.

15. Since there was little differences in the ratio of various metabolites between the two herbicides, the key difference in metabolism was the initial detoxication reaction involving the parent compound.

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APPENDICES

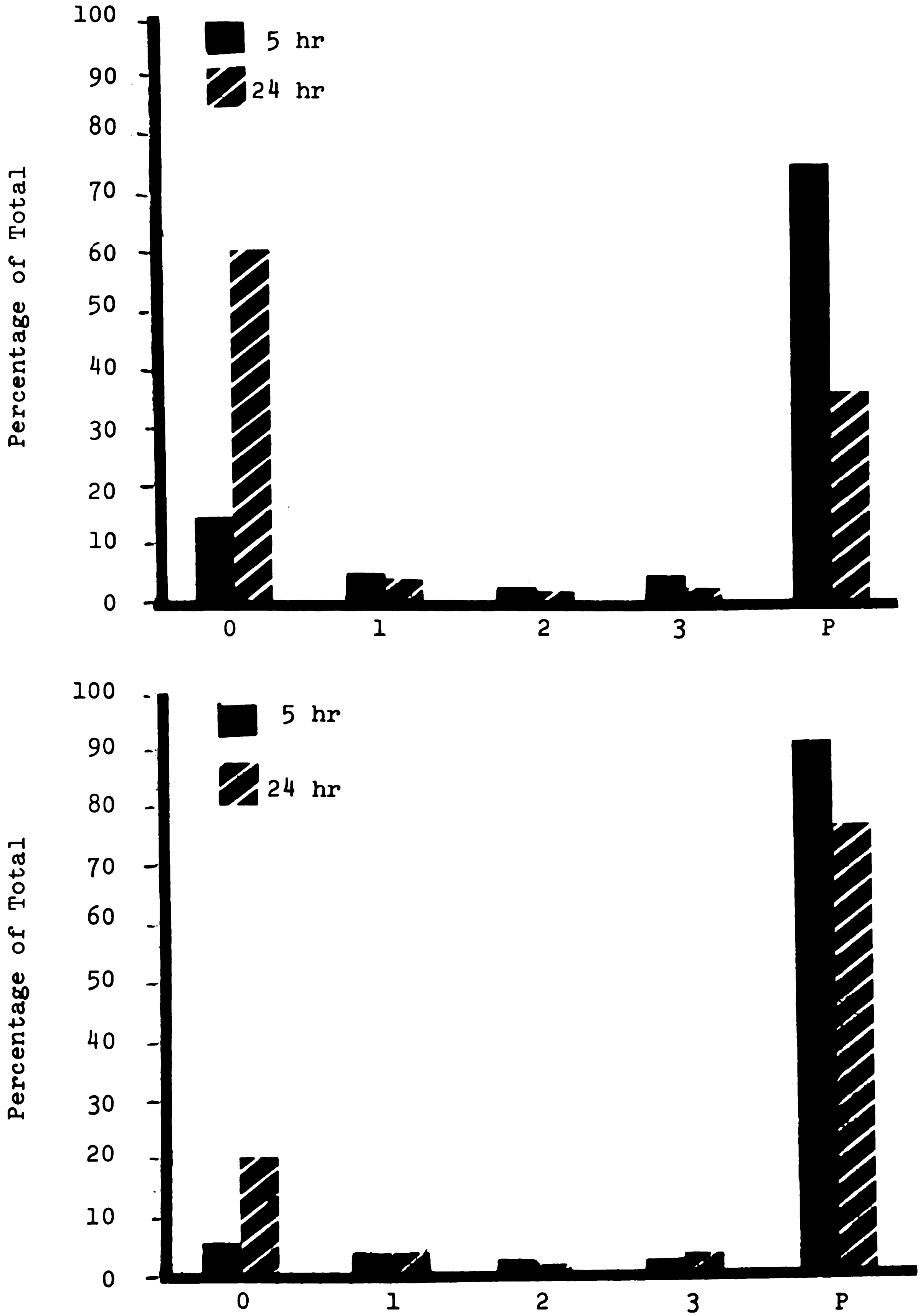
APPENDIX A

Method of recorder chart paper conversion to ug/CO_2/min.

Compressed air flow rate	500 cc/min
CO_2 content of compressed air	330 ppm or .033 percent
Molecular weight of CO_2	44
Standard volume	22.4 l/mole

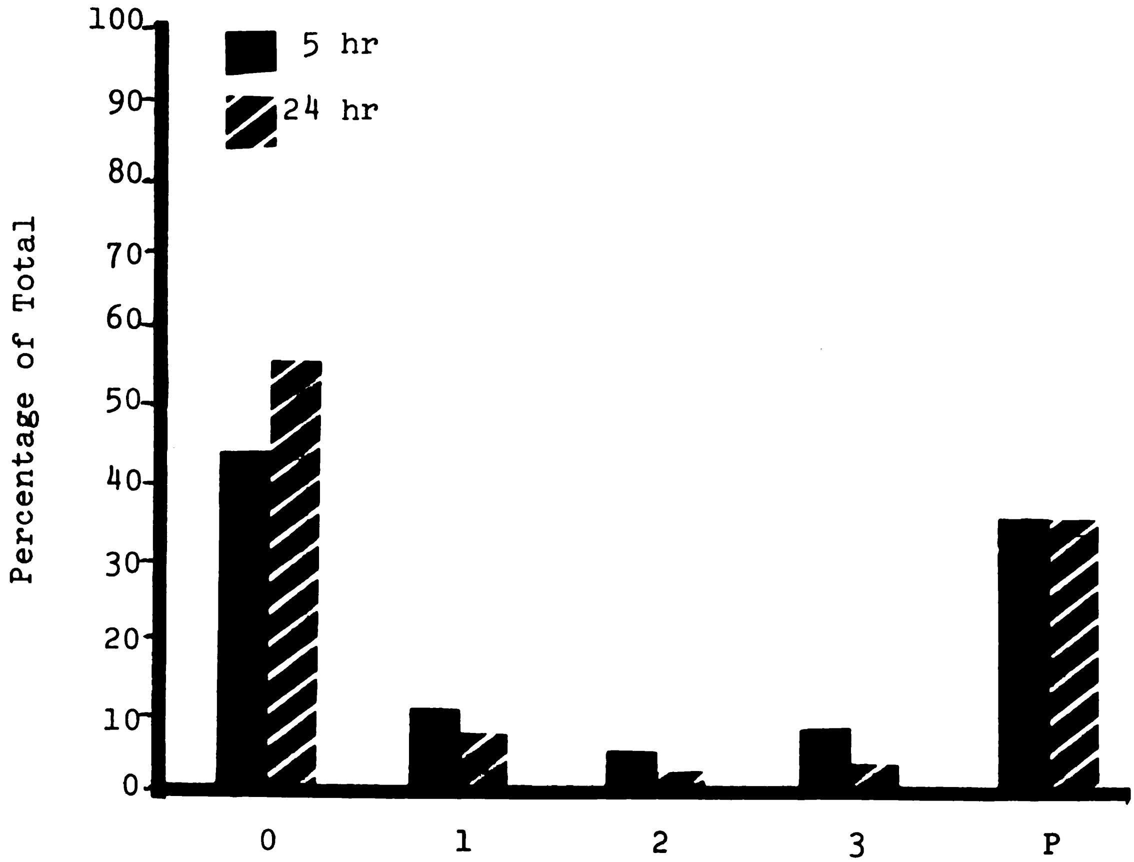
22.4 l contains 1 mole of gas
.5 l/min contains .0223 M (g/l) of gas/min
1 mole of gas contains .033 percent CO_2
therefore .0223 M/min contains 7.359×10^{-6} M CO_2/min
or $7.359 \times 10^{-6} \times 44 = 3.23 \times 10^{-3}$ g CO_2/min
or 3.23 mg CO_2/min = 323 ug CO_2/min
therefore on Beckman[R] #101283 recorder paper 1 unit
change = 6.46 ug CO_2/min

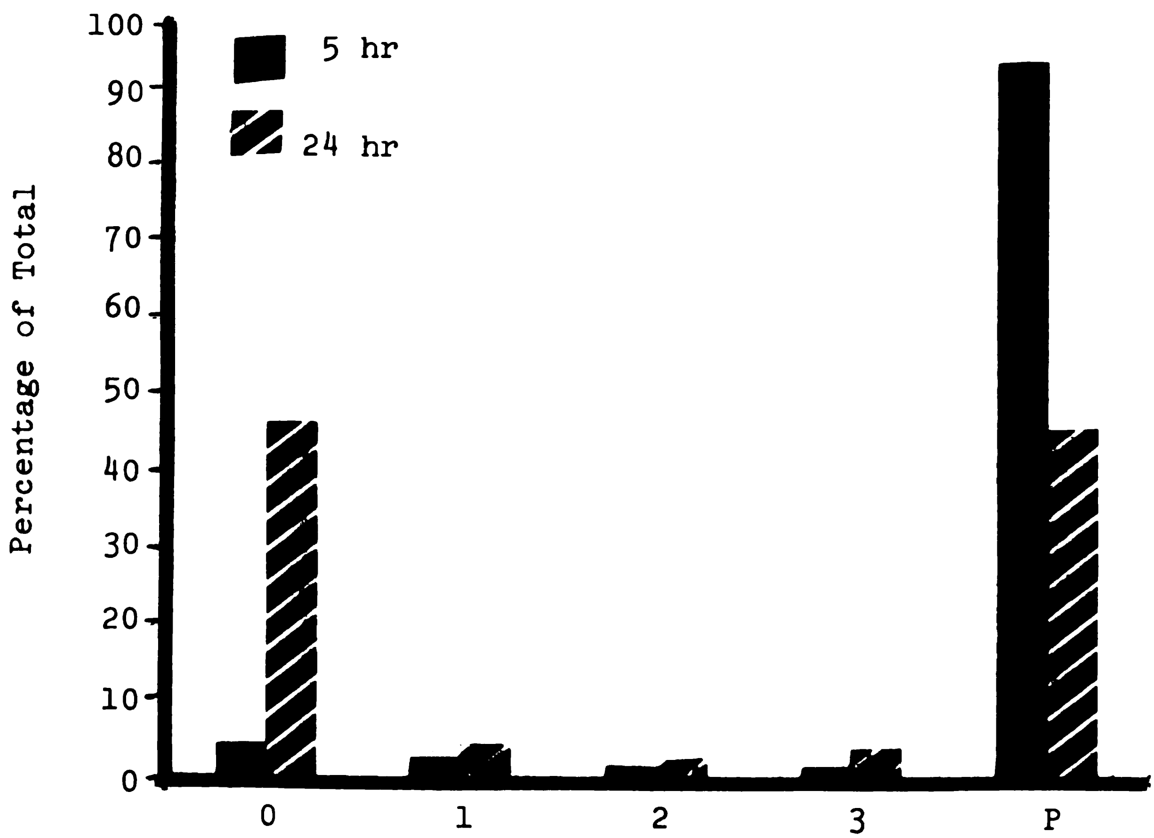
APPENDIX B-1



Chromatographic separation of labelled acetone soluble extracts from phenmedipham (top) and EP-475 (bottom) treated wild mustard using solvent system I. Rf for O(origin)=0.00-0.04; 1=0.04-0.11; 2=0.11-0.20; 3=0.20-0.35; P(parent)= 0.35-0.48.

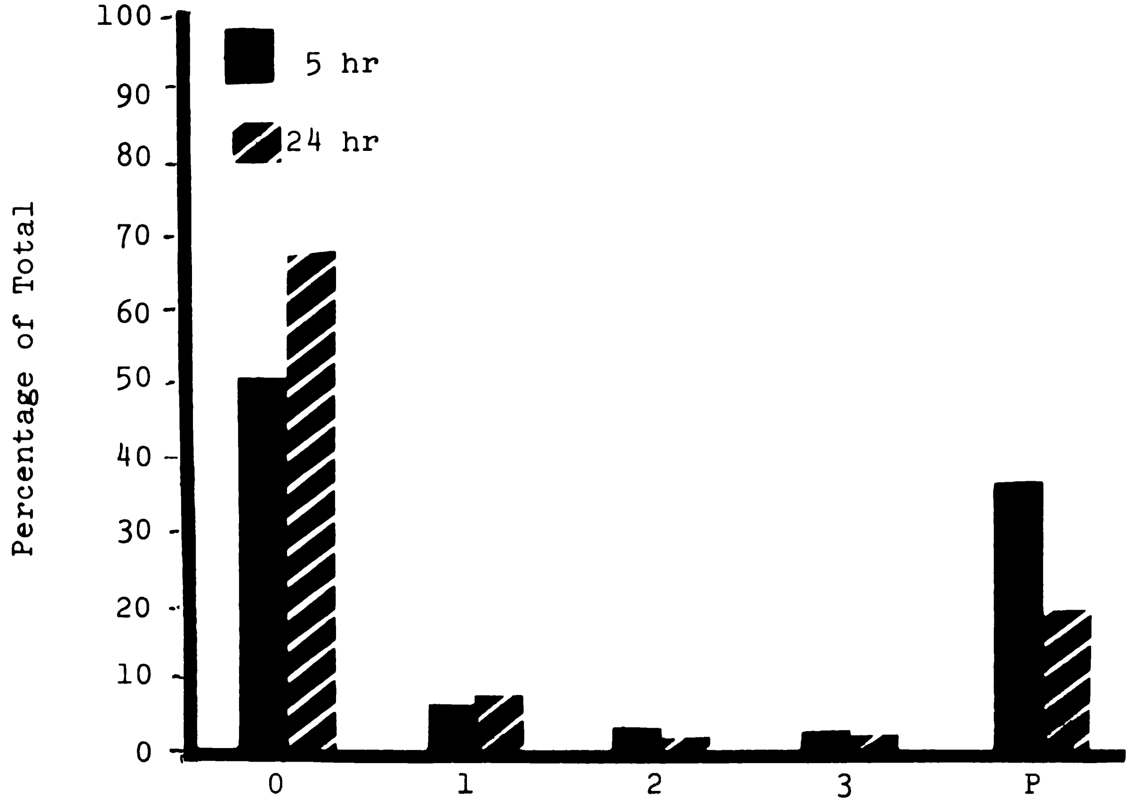
APPENDIX B-2

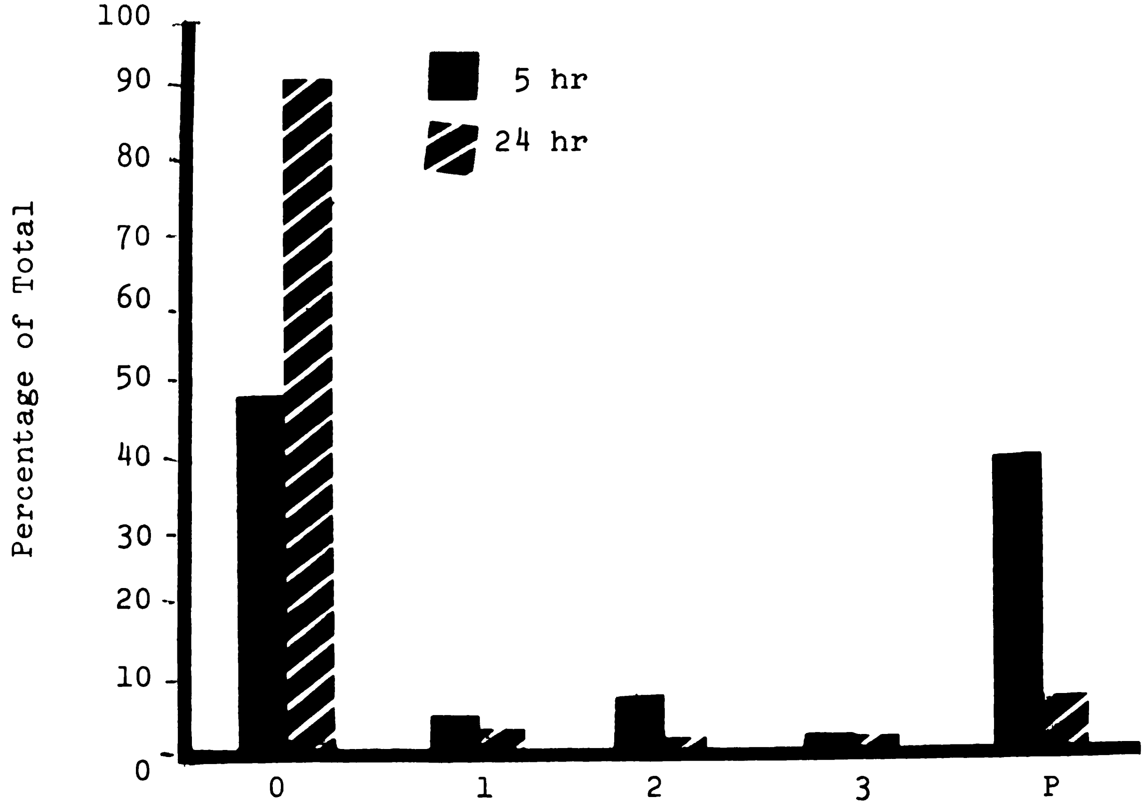




Chromatographic separation of labelled acetone soluble extracts from phenmedipham (top) and EP-475 (bottom) treated redroot pigweed using solvent system I. Rf for 0(origin)=0.00-0.04; 1=0.04-0.11; 2=0.11-0.20; 3=0.20-0.35; P(parent)=0.35-0.48.

APPENDIX B-3





Chromatographic separation of labelled acetone soluble extracts from phenmedipham (top) and EP-475 (bottom) treated sugar beet using solvent system I. Rf for 0(origin)=0.00-0.04; 1=0.04-0.11; 2=0.11-0.20; 3=0.20-0.35; P(parent)=0.35-0.48.