SELECTIVITY AND FATE OF 2 - CHLORO - N - (1 - METHYL - 2 - PROPYNYL) ACETANILIDE (PRYNACHLOR) IN ONION AND PROSO MILLET

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ABSTRACT

SELECTIVITY AND FATE OF 2-CHLORO-N-(1-METHYL-2-PROPYNYL) ACETANILIDE (PRYNACHLOR) IN ONION AND PROSO MILLET

By

Edward Louis Silvia

Preemergence applications of 2-chloro-N-(1-methyl-2-propynyl) acetanilide (prynachlor) effectively controlled annual grasses and selected broadleaved weeds up to 6 weeks in muckland onions (Allium cepa L. 'Downing Yellow Globe'). All rates provided commercially acceptable control for 3 weeks and longer term weed control was obtained at higher rates. Prynachlor was highly effective in controlling all weeds present with the exception of Pennsylvania smartweed (Polygonum pensylvanicum L.). Preemergence or postemergence applications at the loop, flag, and one leaf stages at rates as high as 6.72 kg/ha produced no significant reduction in onion dry weights. Greenhouse rate studies showed that on muck soil, prynachlor inhibited growth of onion less than 50% at rates as high as 11.2 kg/ha, whereas onions were killed at 6.72 kg/ha on a mineral soil mix. In contrast, susceptible proso millet (Panicum miliaceum L.) was killed at 1.12 kg/ha on muck soil and 0.56 kg/ha on mineral soil mix.

Studies were conducted to determine the basis for selectivity in these two monocotyledonous plants. Root and shoot uptake of ^{14}C-prynachlor was assayed utilizing emerging seedlings and a micro split-pot technique. After 18 hours, uptake by proso millet roots exceeded that of onion roots by 58%. However, over longer time periods, onion absorbed more than proso millet. Root uptake was greater than shoot

uptake in both species when equal amounts of herbicide were applied to each zone. The amount transported from root to shoot in proso millet was up to 20 fold that which occurred in onions. Germinating onion seedlings metabolized prynachlor more rapidly than proso millet seedlings, although similar metabolites were found in both species. In 3 week old plants, uptake by onion exceeded that of proso millet and majority of the activity remained in the roots of both species. Onion roots reached maximum uptake in 24 hours, whereas activity in proso millet roots continued to increase throughout 96 hours. Rapid metabolism of prynachlor was accomplished by root and shoot of onion. Over 96% was converted to non-toxic, 2-oic-N-(1-methyl-2-propynyl) acetanilide. Less than 2% of the activity was identified as parent compound. A similar distribution of metabolites was observed in root and shoot of proso millet.

^{14}C-prynachlor was applied preemergence to onions growing on muck soil to follow residue levels until harvest. At the flag stage, nearly 10 fold higher concentrations of ^{14}C-compounds were detected in the onion shoots than in the roots. At maturity, the concentration of ^{14}C-compounds detectable in the onion bulbs was about 1 part per billion expressed on a dry weight basis.

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CHAPTER I

LITERATURE REVIEW

Chemical Weed Control in Onions (*Allium cepa* L.)

Weeds are a major problem in onion production. They may smother relatively slow growing seedling onions, and compete for nutrients and water. On high organic matter soils, weed control is made more difficult by the numerous flushes of weeds throughout the season and the short-lived activity of most herbicides. Dense populations of weeds and diverse species also amplify the problem. On Michigan mucklands, the major annual weed species are large crabgrass (*Digitaria sanguinalis* (L.) Scop.), yellow foxtail (*Setaria glauca* (L.) Beauv.), barnyardgrass (*Echinochloa crusgallis* (L.) Beauv.), witchgrass (*Panicum capillare* L.), redroot pigweed (*Amaranthus retroflexus* L.), common purslane (*Portulaca oleracea* L.), common chickweed (*Stellaria media* (L.) Cyrillo), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), common lambsquarters (*Chenopodium album* L.), and mustards (*Brassica sp.*).

The earliest method of combatting weeds in onions was hand weeding and cultivation. On muck soils, this is extremely difficult without reducing onion stands and also is extremely costly. It was estimated that hand weeding of onions may require as much as 170 hours of labor per acre (24). In hopes of reducing the high labor requirements for hand weeding, chemical weed control was initiated in the 1940's. It was recognized that morphological features of onions such as waxy leaves, and their tendency to grow erect might lend tolerance to the crop.

One of the first chemicals to be utilized based on this type of selectivity was sulfuric acid. It could be sprayed over the tops of onions with minimum damage to crop and burn off most of the emerged weeds (12). Although control was effective, sulfuric acid was harsh on men, equipment, and perhaps soils. Several oils such as diesel oil and stoddard solvent were also found to be effective, but caused yield reductions. Less crop damage was obtained with amine formulations of (2, 4-dichlorophenoxy) acetic acid (2, 4-D) than with oils (12). Today, these early postemergence herbicides have been replaced by 2, 4-dichlorophenyl -p-nitrophenyl ether (nitrofen), and 3-[p-(p-chlorophenoxy) phenyl]-1,1-dimethylurea (chloroxuron). Applied postemergence to weeds, nitrofen and chloroxuron effectively control many broadleaved weeds and selected annual grasses (8, 22, 30).

The value of preemergence herbicides has not been overlooked in onion production. During the 1950's, two of the more effective preemergence chemicals were 3-(p-chlorophenyl)-1, 1-dimethylurea (monuron) and isopropyl m-chlorocarbanilate (chloropropham) (12). Chloropropham is still recommended for use today (26).

Several of the 2-chloroacetamide herbicides have shown promise for selective preemergence use in vegetable crops (4, 7, 23). The first to be commercially introduced was N,N-dially-2-chloroacetamide (CDAA). It was found to provide good control of annual grasses in particular, and is still in use (7, 26).

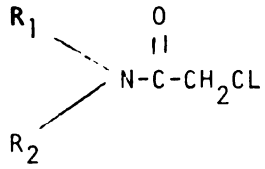
In 1965, 2-chloro-N-isopropylacetanilide (propachlor) was introduced as a preemergence herbicide for control of a broad spectrum of broadleaved weeds and grasses. Propachlor was found superior to several

herbicides in controlling weeds in onions (23, 30). Long-season control on onions was obtained when propachlor was applied preemergence and when chloroxuron was applied postemergence (30). Unfortunately, registration for propachlor as a herbicide for onions was never obtained.

A newer member of the 2-chloroacetamide group, 2-chloro-N-(1-methyl-2-propynyl) acetanilide (prynachlor) has been reported to be highly toxic to both broadleaved weeds and annual grasses, while onion is highly tolerant (4, 27, 29).

Action of the 2-chloroacetamides

General Properties and Structure. The 2-chloroacetamide herbicides belong to a class of herbicides generally known as the amides. They are also known as acetanilides if one substituent group is a phenyl ring. Their properties are varied depending on their substitution. They have a general structure of the following configuration:



Uptake. Researchers have shown that the 2-chloroacetamides are readily taken up by several plant species (1, 4, 9, 10, 11, 16).

Smith et al. (31) attempted to describe the possible relationship between amount of uptake of 2-chloroacetamide derivatives and susceptibility. Germinating seeds of corn (Zea mays L.) and soybean (Glycine max L.) represented tolerant species, whereas oats (Avena sativa L.) and cucumber (cucumis sativus L.) represented susceptible species.

All of the test plants absorbed the 2-chloroacetamide derivatives, but to different extents. Corn absorbed the least and soybean the greatest amount of the chemicals. It was concluded that susceptibility was not determined by the amount of the chemical absorbed.

Split-pot experiments showed that site of uptake of 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) in cotton (Gossypium hirstum L.) was directed toward the root zone rather than the shoot zone. Cotton was treated with alachlor at the root zone, shoot zone, and both root and shoot zone. When absorption of the chemical was in the root zone, dry weight of the cotton plants were reduced considerably. Only a slight reduction in dry weights occurred when soil in the shoot zone was treated, and placing the chemical in both root and shoot zones only slightly increased phytotoxicity (11).

Knake et al. (18) reported that when giant foxtail (Setaria faberii Herrm.) was treated with alachlor in the shoot or root zone, it was the shoot zone treatment which effected the plant most noticably, leading one to believe that the major site of uptake in monocotyledons is the shoot zone.

Armstrong (1) showed that application of alachlor to the roots of yellow nutsedge (Cyperus esculentus L.) did not reduce plant growth, whereas application in the shoot zone gave excellent control.

Metabolism. Metabolism of the 2-chloroacetamides has been studied by several researchers (10, 15, 19, 31, 32). Studies of CDAA metabolism in corn and soybean showed that CDAA was completely metabolized in both species within four days (16). Co-chromatographic analysis of the extracts of corn confirmed glycolic acid as the major metabolite.

In soybean seedlings, glyoxylic rather than glycolic acid was found to be the major product of CDAA metabolism. Research by Zelitch and Ochoa showed glyoxylic acid to be in equilibrum with glycolic acid, both of which function in plant respiration (35, 36).

In corn and soybean, propachlor has been found to be rapidly metabolized to a highly polar metabolite (17). Through hydrolysis and vapor-phase chromatography the metabolite was found to be a water-soluble acidic compound with a structure similar to the parent compound, minus the chloro group. This is believed to have been displaced by some nucleophilic endogenous substrate in the plant (25). Lamoureux et al. (19) found propachlor to be metabolized by corn to both the glutamyl-cysteine conjugate and the glutathione conjugate of propachlor. With 3',4'-dichloropropionanilide (propanil), Stroller et al. (33) reported that the parent compound is first converted to arylamines and then complexed with glucose.

Selectivity. Smith et al. (31) studied the rate of metabolism of 2-chloroacetamide derivatives by both tolerant and susceptible plants. He found that both susceptible and tolerant test plants could absorb and metabolize the chemical in 48 hours, but in 6 hours tolerant species were able to metabolize larger amounts, whereas susceptible species metabolized very little. It was concluded that the degree of tolerance of various seedlings to 2-chloroacetamides seems to be related to the length of time required to metabolize the chemical. Those species which metabolized the chemical as soon as it entered or within a short time thereafter, had only small amount of parent compound present. In contrast, susceptible species incapable of rapid metabolism accumulated higher and therefore, lethal concentrations.

The mechanism of selectivity for propanil was proposed by Still et al. (32). He suggested that the tolerance of rice is correlated with the activity of acylanilide hydrolase an enzyme which deactivates propanil.

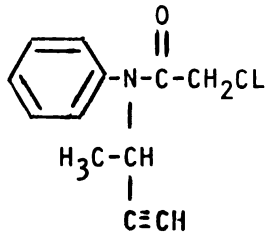
Mode of Action. The 2-chloroacetamides are believed to inhibit growth by stopping cellular elongation and cell division. Cavin et al. (5) showed that when CDAA was applied to susceptible barley (Hordeum vulgare L.) and peas, (Pisum sativum L.) cell division was inhibited in germinating seedlings, and the amount of inhibition was directly proportional to the concentration of CDAA. Propachlor possesses similar cellular inhibition characteristics (3, 9, 10). Dhillon et al. (9) reported that cell division in onion root was totally inhibited by propachlor at 16 ppm.

A possible mode of action of CDAA was postulated by Jaworski (16) who reported that CDAA reduced the respiration of germinating rye grass (Lolium multiflorum Lam.) by inhibiting sulfhydryl containing enzymes involved in respiration. Studies with thiol compounds and the 2-chloroacetamides showed an interaction with sulfhydryl groups and various enzyme system (20). Mann et al. (21) treated barley coleoptiles with CDAA and found 51 to 70% inhibition of ^{14}C-leucine incorporation into protein at 2 and 5 ppm respectively. He concluded that CDAA was either inhibiting the uptake of certain amino acids or causing inhibition of protein synthesis.

In work with propachlor, Duke et al. (10) reported inhibition in cucumber roots and correlated this with the inhibition of protein synthesis in root tips. He further suggested that the primary site of action is at the level of protein formation and is due to the prevention of the transfer of aminoacyl-sRNA to the polypeptide chain.

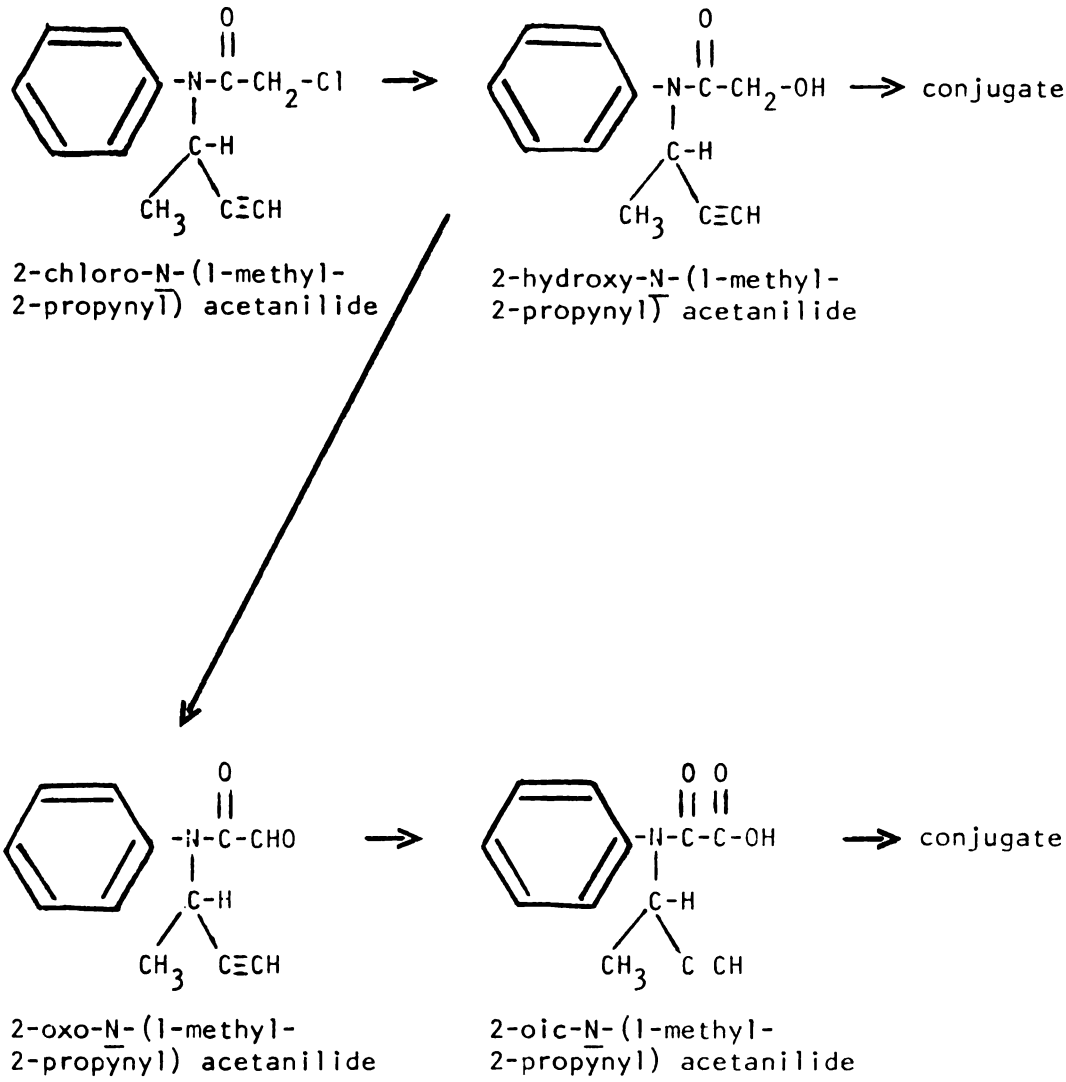
Prynachlor

Structure and Properties. Prynachlor is a member of the 2-chloroacetamide group and has a phenyl ring and a (1-methyl, 2-propynyl) group fulfilling the N-substitution. It has the following structure:



Prynachlor has a molecular weight of 221.7, is highly soluble in benzene, and its solubility in water and ethanol at 20°C is 0.50 and 239.2 g/L., respectively (34). Its melting point is 40-47°C. Acute toxicity tests indicated an oral LD_{50} of 1177 mg/kg for rats and a dermal LD_{50} of 1926 mg/kg for rabbits (2).

Metabolism. Prynachlor metabolism studies in corn and soybean have found the majority of the metabolite present to be the conjugated acid, 2-oic-N-(1-methyl-2-propynyl) acetanilide (28). Metabolism involves hydroxylation at the chlorine position followed by several oxidation steps. The metabolic degradation scheme for prynachlor in corn as proposed by Hazelton Laboratories is given on page 8.


-N-C-CH2-Cl
-N-C-CH2-OH
conjugate
C-H
CH3
CΞCH
2-chloro-N-(1-methyl-2-propynyl) acetanilide
2-hydroxy-N-(1-methyl-2-propynyl) acetanilide
-N-C-CHO
-N-C-C-OH
C CH
2-oxo-N-(1-methyl-2-propynyl) acetanilide
2-oic-N-(1-methyl-2-propynyl) acetanilide

CHAPTER 2

Herbicidal Activity of Prynachlor on Muckland Onions

Materials and Methods

Preemergence tests. Field studies were conducted during 1971 and 1972 at the Michigan State University Muck Experimental Farm, on a Houghton muck (80% organic matter) with a resident population of common purslane, redroot pigweed, Pennsylvania smartweed, large crabgrass, barnyardgrass, witchgrass, and yellow foxtail. The field was plowed, fertilized, disk harrowed, and floated after which onions 'Downing Yellow Globe' were seeded on May 19, 1972. The rows were spaced 30 cm apart with 50 seeds per meter. Prynachlor was applied on plots 1.22 X 7.63 m with a CO_2 powered plot sprayer regulated at 30 psi. Prynachlor (4EC) was applied preemergence at rates of 0, 2.24, 3.36, 4.48, 6.72 and 8.96 kg/ha. Each treatment was replicated four times in a randomized complete block design. Plots were evaluated for both broadleaved weed and annual grass control on a 1 to 9 scale where 1=no control and 9= complete control. Weed ratings were obtained 3 and 6 weeks after initial seeding. Control plots were hand weeded once a week until harvest. Eight weeks after treatment, prynachlor treated plots were hand weeded along with control plots. At maturity, the crop was hand-harvested and total fresh weights of bulbs from each plot recorded.

Postemergence tests. Crop safety studies were conducted to determine the tolerance of onions to prynachlor at early stages of crop growth. This study was also conducted at the Michigan State University Muck Experimental Farm during the summer of 1971. Onions were seeded into 1.22 X 7.63 m plots at one week intervals for 4 weeks. Plots were hand

weeded until application of the herbicide on the fourth week. Treatments were replicated 3 times in a split-plot design. The herbicide was applied at rates of 0, 2.24, 3.36, 4.48, and 6.72 kg/ha as previously described.

Two weeks after herbicide application, shoot growth from one meter sections of row was cut from each plot. Shoots were oven dried at 45°C and weights recorded to the nearest mg.

Results and Discussion

Preemergence applications of prynachlor effectively controlled annual grass and selected broadleaved weeds up to 6 weeks (Table 1).

Table 1. Response of weeds and onions to preemergence applications of prynachlor.

Prynachlor rate	21 Day		42 Day		
	Broadleaf[1]	Grass	Broadleaf[1]	Grass	Yield
(kg/ha)	(weed control rating)				(kg/ha)
0	1.0	1.0	1.0	1.0	21,138.
2.24	7.7	8.0	6.3	6.0	28,865.
3.36	8.3	8.3	7.5	7.0	29,678.
4.48	9.0	9.0	8.5	7.7	32,931.
6.72	9.0	9.0	9.0	8.0	36,183.
8.96	9.0	9.0	9.0	9.0	37,403.
HSD AT 5% LEVEL	.64	.47	1.28	1.28	11,312.

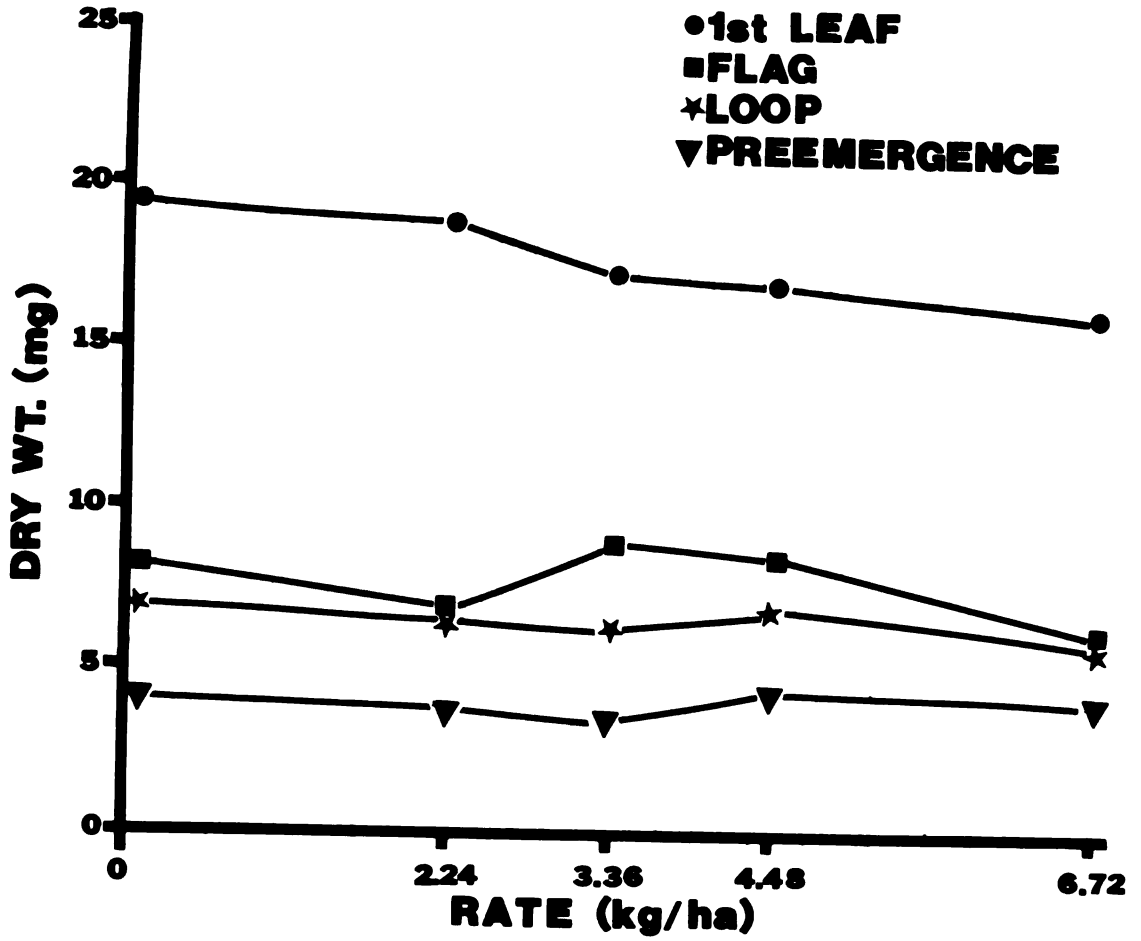
[1]Pennsylvania smartweed not included in rating.

All rates provided commercially acceptable control for 3 weeks, and longer term weed control increased at higher rates. Although an unusually dense weed population (1617 weed/m^2) existed, prynachlor was highly effective in controlling all weeds present with the exception of Pennsylvania smartweed, which consisted of 3% of the population.

An increase in onion yields occurred with increasing rates of the herbicide up to 8.96 kg/ha. This may have resulted because of 1.) the inability to maintain the hand weeded control plots as free of weeds as chemically treated plots, 2.) damage to weeded plots by hoeing and cultivation and 3.) the higher densities of Pennsylvania smartweed at lower rates of prynachlor.

Preemergence or postemergence applications at the loop, flag, and one leaf stages at rates as high as 6.72 kg/ha showed no significant reduction in onion dry weights (Figure 1). This data indicated that prynachlor can be applied safely to emerged onions without injury. However, application on emerged weeds are also ineffective, so fields would have to be cultivated and/or weeded prior to application of prynachlor.

Figure 1. Response of onion seedlings at four stages of growth to prynachlor.


●1st LEAF
■FLAG
★LOOP
▼PREEMERGENCE
DRY WT. (mg)
25
20
15
10
5
0
0
2.24
3.36
4.48
6.72
RATE (kg/ha)

CHAPTER 3

Basis for the Selectivity of Prynachlor in Onion and Proso Millet

Abstract. On a Houghton Muck Soil, 2-chloro-N-(1-methyl-2-propynyl) acetanilide (prynachlor) inhibited growth of onion (Allium cepa L. 'Downing Yellow Globe') less than 50% at rates as high as 11.2 kg/ha. In contrast, onions were killed at 6.72 kg/ha on a mineral soil. Susceptible proso millet (Panicum miliaceum L.) was killed at 1.12 kg/ha on muck soil and 0.56 kg/ha on mineral soil. Root and shoot uptake of ^{14}C-prynachlor was assayed utilizing emerging seedlings and a micro split-pot technique. After 18 hours, uptake by proso millet roots exceeded that of onion roots by 58%. However, over longer time periods, onion absorbed more than proso millet. Root uptake was greater than shoot uptake in both species when equal amounts of herbicide were applied to each zone. The amount transported from root to shoot in proso millet was up to 20 fold that which occurred in onions. Germinating onion seedlings metabolized prynachlor more rapidly than proso millet seedlings.

Introduction

Herbicide selectivity may be due to physical, morphological and/or physiological factors.

Physiological selectivity is determined by rates of uptake, translocation, and biochemical alteration of the herbicide by the plant (5, 14). It is probably the most important type of selectivity for preemergence

herbicides. Researchers have correlated differential uptake and translocation with susceptibility of certain plant species (4, 16). Plants can metabolically alter herbicide activity. Classic examples are the differential dealkylation of 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (atrazine), demethylation of 3-(p-chlorophenyl)-1,1-dimethylurea (monuron), and conjugation of 5-amino-4-chloro-2-phenyl-3 (2H)-pyridazinone (pyrazon) (6, 8, 10, 11, 12). Smith et al. (9) observed uptake of the 2-chloroacetamides by both tolerant and susceptible species, but he could not relate this to selectivity. Absorption of the 2-chloroacetamides is effected by the plant zone of uptake. Root zone of cotton was reported to be the primary site of uptake of 2-chloro-N-isopropylacetanilide (propachlor), while giant foxtail (Setaria faberii Herrm.) was effected most noticably when propachlor was placed in the shoot zone (3). The 2-chloroacetamides are metabolized by both tolerant and susceptible plants, but selectivity may be determined by the rate of deactivation (2). Metabolism must be rapid enough to assure that concentrations capable of inhibiting growth are not accumulated.

Prynachlor has demonstrated excellent herbicidal activity on annual grasses and selected broadleaved weeds with selectivity in several crops. It is particularily effective on soils with a high organic matter content.

The objective of this study was to compare uptake, transport, and metabolism of prynachlor in a tolerant and a susceptible monocotyledonous species to determine the basis for selectivity.

Materials and Methods

Tolerance. Comparative tolerance of onion and proso millet was determined by applying increasing rates of prynachlor to plants growing on two soil

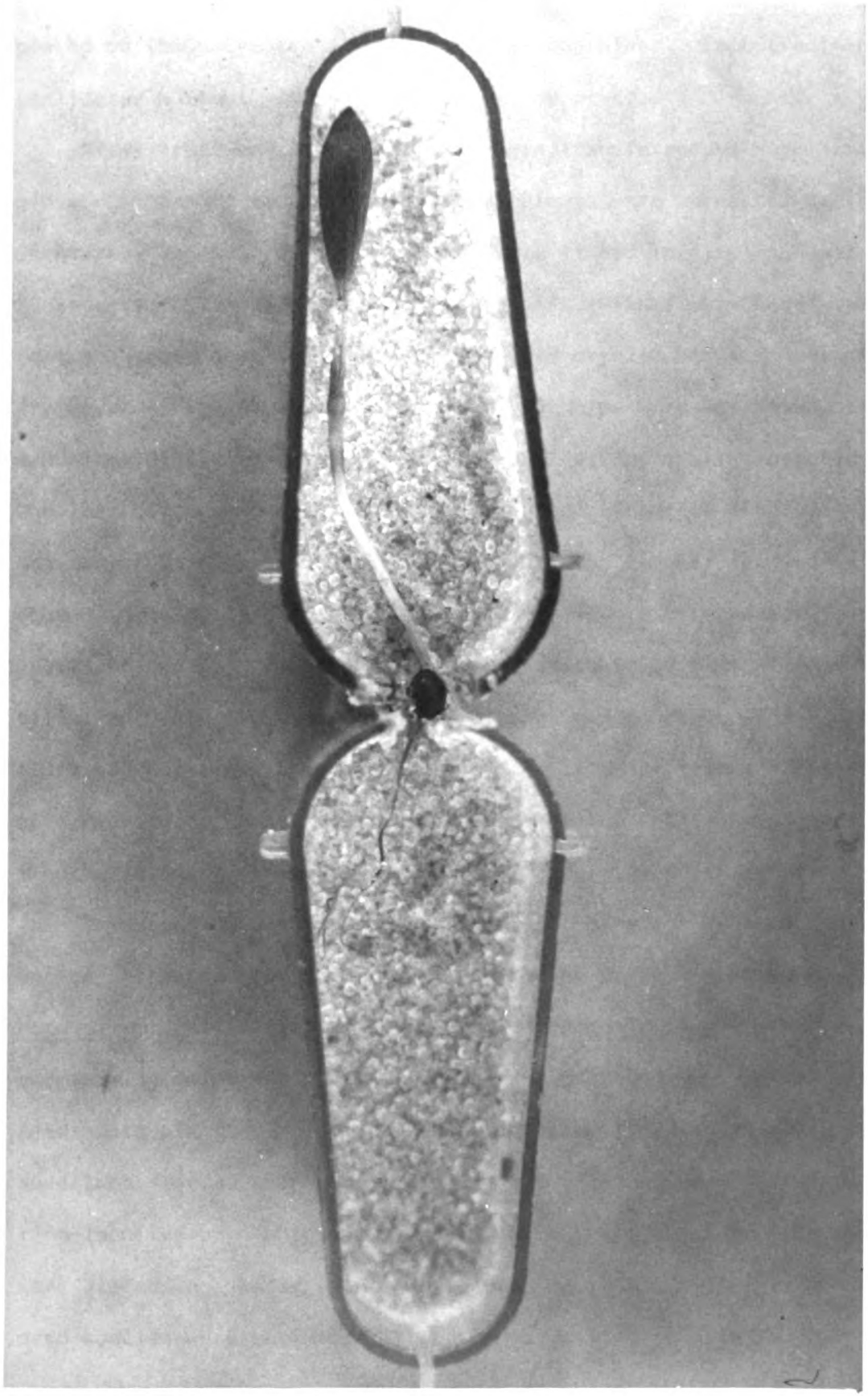
types. Fifty seeds of each species were seeded 1.5 cm deep into 15 by 10 by 7 cm styrofoam flats containing either a Houghton muck soil or mineral soil mix (1 loam: 1 peat: 1 sand). After planting, prynachlor (4EC) was applied with a laboratory sprayer operated at 30 psi to onion at rates of 0, 2.24, 4.48, 6.72, 8.96, and 11.2 kg/ha, and to proso millet at rates of 0, 0.14, 0.28, 0.56, and 1.12 kg/ha, with each treatment being replicated 4 times in a randomized complete block design.

Plants were maintained at a greenhouse night temperature of 21°C and day temperatures ranged from 21 to 32°C. After 2 weeks, aerial growth was collected and dry weights recorded to the nearest 0.1 mg.

Uptake. In order to facilitate study of seedling uptake and translocation of ^{14}C-prynachlor, a micro split-pot was devised (Figure 1). Notches 1 cm deep were cut in plastic nitrogen analyzer vials (10 ml) at the highest end. Two vials were glued together so the cut ends met. Floral putty was placed at the botton of the cut. Nine grams of sterile silica sand was placed in each side.

Plants of both species were seeded in 15 by 10 by 7 cm styrofoam boxes containing vermiculite. The seeds were germinated in a growth chamber (30°C). When plants penetrated the media surface, seedlings were removed for placement in the micro split-pots. A seedling was placed on the floral putty with root portion in one vial and the shoot portion in the other vial after which the notch was filled with putty to provide a barrier between the vials. The seedlings were covered with 4 grams of silica sand. Two ml of distilled water containing 0.21 μc of ring-labelled ^{14}C-prynachlor (4.8 μc/μ mole) was pipetted into either the root or shoot portion of the experimental chamber. Two ml of distilled water was also

Figure 1. Micro split-pot technique utilized to assay uptake of ^{14}C-prynachlor by shoots or roots of onion and proso millet.



placed on the untreated side of the test container. Each treatment was replicated 4 times, and the tests were repeated.

After treatment, the micro pots were transferred to high humidity glass chambers to reduce evaporation. Plants were harvested at 18 and 96 hours. Roots of harvested plants were rinsed in distilled water for 30 seconds to remove the adhering chemical. Plants were separated into root and shoot and immediately frozen with dry ice and acetone. After drying at 45°C, weights were obtained. Samples were combusted utilizing a Nuclear Chicago Model 3151 Oxidizer unit equipped with magnetic stirrers. One liter flasks were purged with oxygen and stoppered with rubber septum caps prior to combustion. Upon cooling of the flasks, 10 ml of ethanol: ethanolamine (2:1 V/V) was injected and they were stirred for 15 minutes. One ml of the CO_2 trapping solution was added to 10 ml of liquid scintillation fluid (4g BBOT/liter of toluene) and counted. The scintillation fluid had 63% efficiency as determined by External Standardization on a Packard Tricarb Scintillation Spectrometer. All cpm (counts per minute) data were converted to dpm (disintegrations per minute).

Transport was determined by dividing the amount of activity from on the untreated side of the seedling by the total activity in the plant.

Metabolism. Metabolism was also studied by applying ^{14}C-prynachlor to germinating onion and proso millet seedlings. One hundred and fifty seeds were planted into styrofoam cups filled with silica sand. When the seedlings started to emerge, 10 ml of distilled water containing 1 μc of ring-labelled prynachlor was pipetted over the surface of each cup. Cups were placed in a water saturated chamber to reduce evaporation. Plants from each species were harvested 18 and 96 hours after treatment and handled

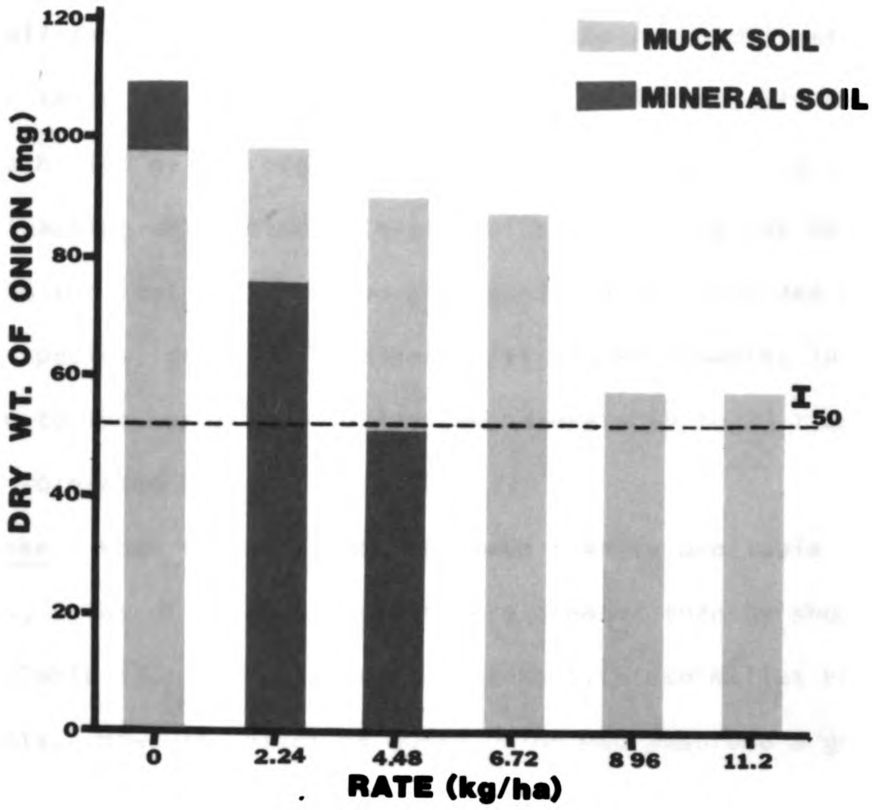
as described in the previous study.

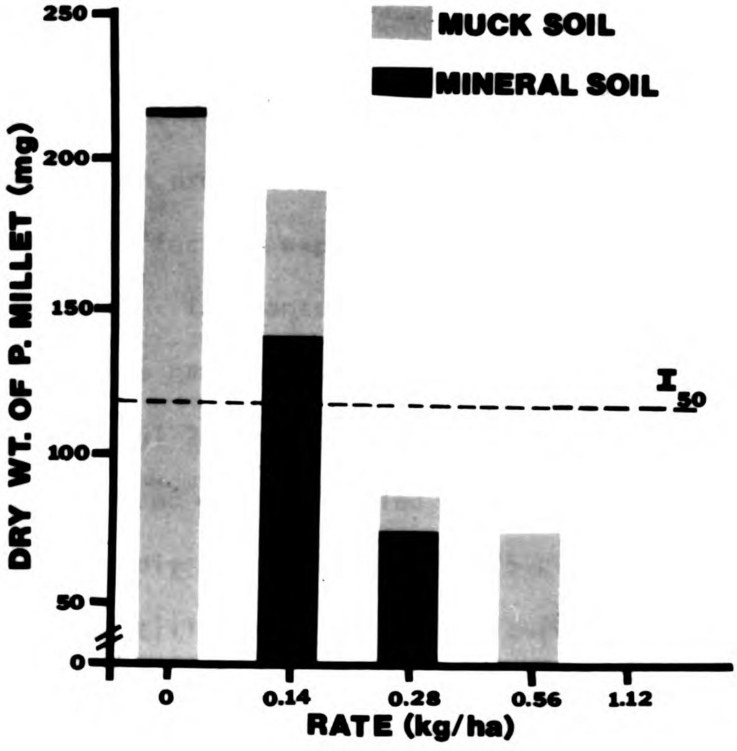
Dried plant portions (100 mg) were ground in a tissue homogenizer and extracted with 6 ml of methanol. Extracts were spun in a clinical centrifuge at 7000 rpm for 20 minutes. The supernatant was then carefully decanted and saved. The pellet was washed with 2 ml of methanol and respun. This procedure was repeated 3 times and the supernatants combined. The methanol extracts were concentrated to 0.5 ml and 100 µl applied as a streak to 250 micron silica gel H thin layer plates. The plates were developed in non-polar and polar solvent systems which were benzene:methanol (97:3 V/V) and methanol:ethyl ether: acetic acid (6:3:1), respectively. The solvent fronts were allowed to run 15 cm. Parent compound and metabolites were co-chromatographed with standards supplied by BASF Wyandotte Corporation, Parsippany, New Jersey. Standards were detected by a bromocresol green-bromophenol blue-potassium permanganate spray reagent. After detection and determination of Rf values for standards, 1 cm sections were scrapped into scintillation vials containing 10 ml of scintillation fluid and counted.

Results and Discussion

Tolerance. In onion, 50% inhibition was reached at 4.48 kg/ha and complete kill was observed at 6.72 kg/ha on mineral soil (Figure 2). On muck soil, a reduction in growth occurred with increasing rates, but onion did not reach 50% inhibition at rates as high as 11.2 kg/ha. Proso millet on mineral soil showed 50% inhibition at 0.28 kg/ha. Complete kill was accomplished at 0.56 kg/ha on mineral soil and 1.12 kg/ha on muck soil.

Figure 2. Response of a.) onions and b.) proso millet growing on two soil types to preemergence application of prynachlor.


MUCK SOIL
MINERAL SOIL
DRY WT. OF ONION (mg)
120
100
80
60
40
20
0
I_{50}
0
2.24
4.48
6.72
8 96
11.2
RATE (kg/ha)


MUCK SOIL
MINERAL SOIL
DRY WT. OF P. MILLET (mg)
250
200
150
100
50
0
I_{50}
0
0.14
0.28
0.56
1.12
RATE (kg/ha)

At all rates, both species growing on the muck soil were injured less than those growing on mineral soil. This is probably due to binding of the herbicide by the organic matter which reduces its activity, and greater leaching which occurs in mineral soils moving the herbicide more rapidly to the root zone. Although organic matter provided protection for both species, great differences exist between species in inherent tolerance to the herbicide. Onion can tolerate 10 to 20 times the rate which is tolerated by proso millet.

Uptake. When the herbicide was made readily available to germinating seedlings, rates of uptake by roots were greater than by shoots of both species (Table 1). After 18 hours, uptake by proso millet roots exceeded onion roots. However, after 96 hours onion had absorbed a greater quantity of the herbicide. Shoots of onion more effectively absorbed prynachlor than proso millet shoots. In proso millet, a higher percentage of that absorbed by the root was transported to the shoot (Table 2). In both species, basipetal transport was limited.

Although the increased rate of accumulation and greater transport of prynachlor in proso millet may contribute to susceptibility, they may not be the only factors explaining the basis for selectivity.

Metabolism. In plants exposed 18 hours to ^{14}C-prynachlor, onion roots and shoots had metabolized 96% of the radioactivity to non-toxic 2-oic-N-(1-methyl-2-propynyl) acetanilide (Figure 3). Only 2% of the activity recovered was identified as parent prynachlor. Proso millet roots also rapidly metabolized the herbicide, but after 18 hours, proso millet shoots still contained 24% unaltered prynachlor.

Table 1. Uptake of ^{14}C-prynachlor by roots and shoots of onion and proso millet.

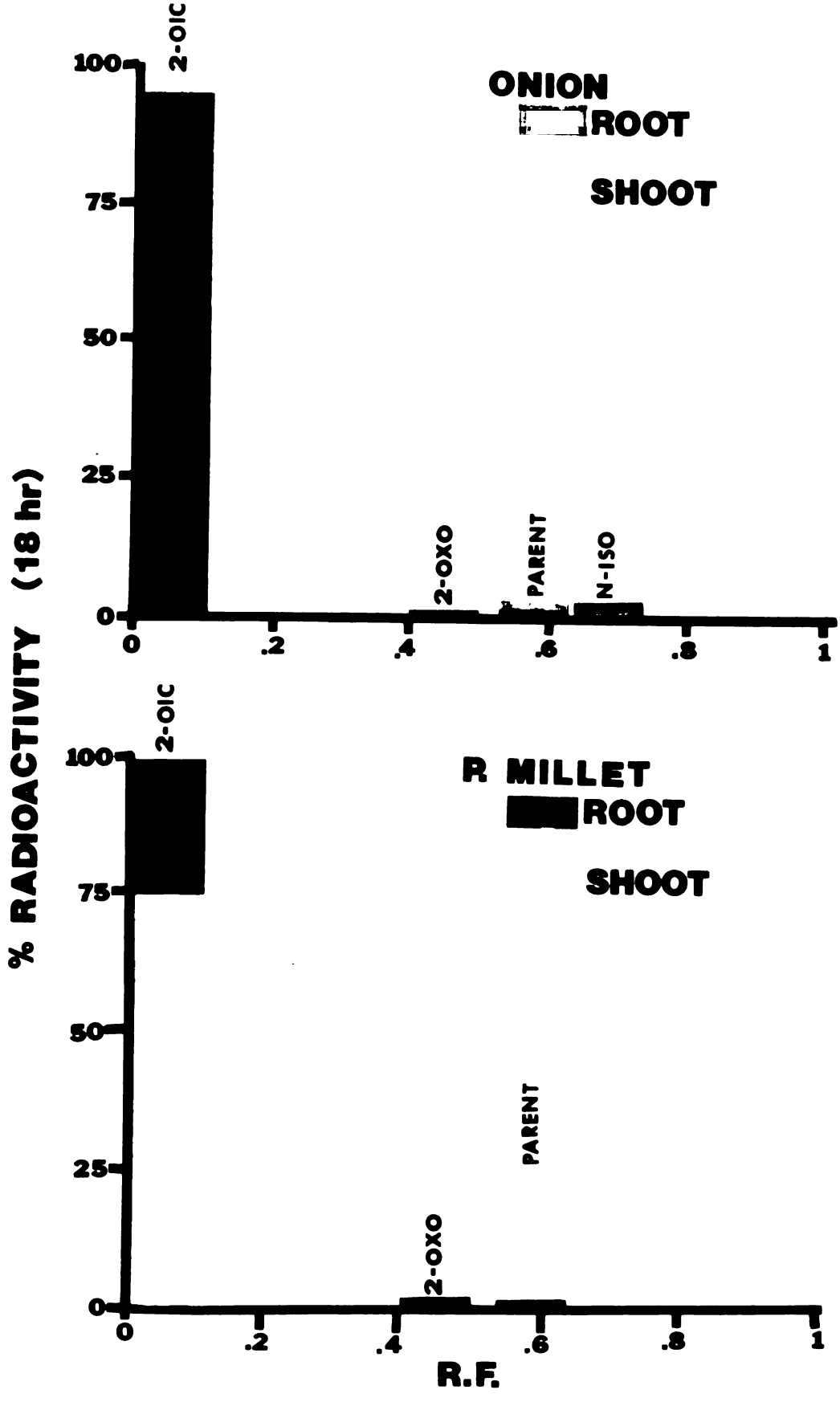
Plant	Portion exposed	Total uptake		Mean[1]
		18 hr.	96 hr.	
		(dpm)		
Onion	Root	908	2153	1531
	Shoot	595	1838	1217
	Mean[1]	752	1996	
Proso millet	Root	1431	1816	1623
	Shoot	374	1280	827
	Mean[1]	903	1548	

[1]Interaction of species X portion and species X time is significant at at the 5% level.

Table 2. Transport of ^{14}C-prynachlor in onion and proso millet after root or shoot exposure.

Plant	Portion exposed	% Transported	
		18 hr.	96 hr.
Onion	Root	0.4	5.4
	Shoot	1.1	4.2
Proso Millet	Root	5.8	11.6
	Shoot	1.7	1.6

Figure 3. Distribution of ^{14}C-prynachlor (parent), 2-oic-N-(1-methyl-2-propynyl) acetanilide, 2-oxo-N-(1-methyl-2-propynyl) acetanilide, and N-isobutynyl aniline in onion and proso millet after 18 hours.


ONION
ROOT
SHOOT
2-OIC
2-OXO
PARENT
N-ISO
R MILLET
ROOT
SHOOT
2-OIC
PARENT
2-OXO
% RADIOACTIVITY (18 hr)
100
75
50
25
0
0
.2
.4
.6
.8
1
R.F.

In a polar solvent system, 2-oic-N-(1-methyl-2-propynyl) acetanilide was found to move readily and co-chromatograph with the standard (Figure 4). Small quantities of metabolites were also detected that co-chromatographed with 2-hydoxy-N-(1-methyl-2-propynyl) acetanilide, 2-oxo-N-(1-methyl-2-propynyl) acetanilide and N-isobutynyl aniline standards. Although less than 2% parent compound was present in both onion and proso millet after 96 hours (Figure 5), germinating onion seedlings metabolized ^{14}C-prynachlor at a faster rate than proso millet seedlings.

The process of rapid metabolism in onion may prevent the accumulation of toxic levels of prynachlor which imparts tolerance. This is in agreement with the explanation offered by Jaworski on selectivity of other 2-chloroacetamides.

Figure 4. Development of 2-oic-N-(1-methyl-2-propynyl) acetanilide in a polar solvent system.

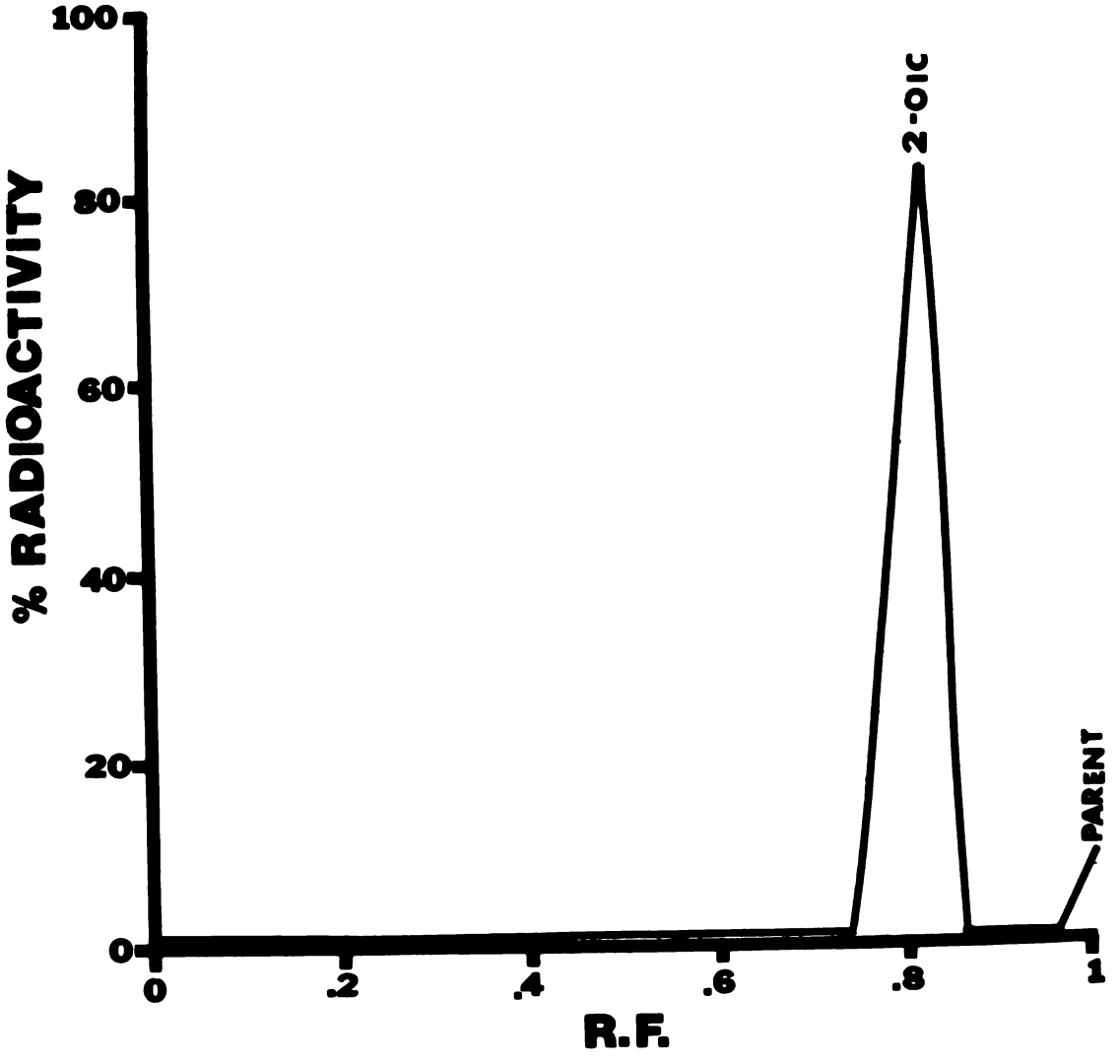
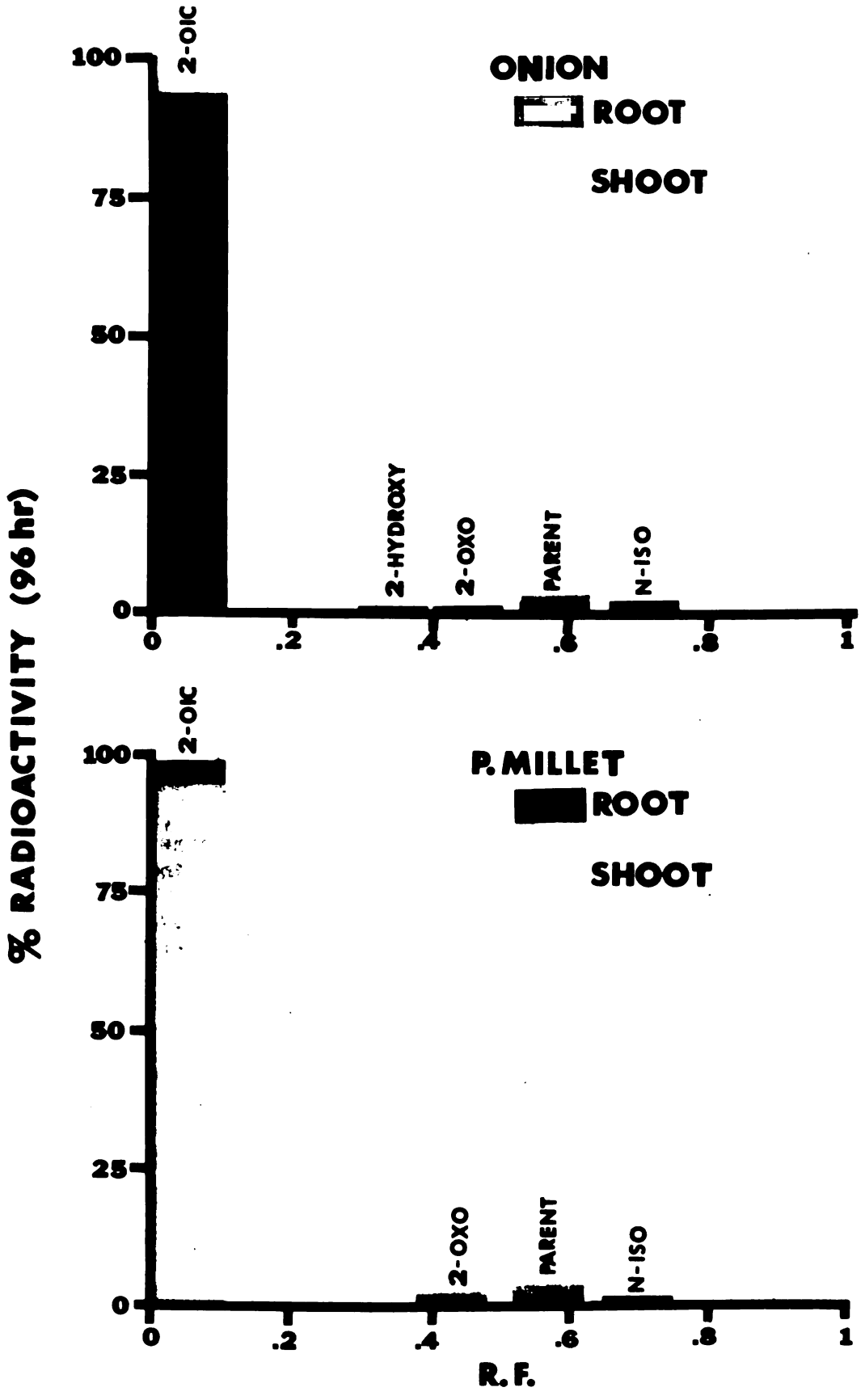

100
80
60
40
20
0
% RADIOACTIVITY
2-OIC
PARENT
0
.2
.4
.6
.8
1
R.F.

Figure 5. Distribution of ^{14}C-prynachlor (parent), 2-oic-N-(1-methyl-2-propynyl) acetanilide, 2-hydroxy-N-(1-methyl-2-propynyl) acetanilide, 2-oxo-N-(1-methyl-2-propynyl) acetanilide, and N-isobutynyl aniline in onion and proso millet after 96 hours.


ONION
ROOT
SHOOT
2-OIC
2-HYDROXY
2-OXO
PARENT
N-ISO
P. MILLET
ROOT
SHOOT
2-OIC
2-OXO
PARENT
N-ISO
% RADIOACTIVITY (96 hr)
R.F.
100
75
50
25
0
.2
.4
.6
.8
1

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CHAPTER 4

Fate of Prynachlor in Maturing Onion Plants

Materials and Methods

Residue study. Houghton Muck soil was collected from the Michigan State University Muck Experimental Farm in the fall of 1971 from an area with no previous pesticide use. The soil was potted in 3 styrofoam cups which were 6.7 cm in diameter with an approximate volume of 190 cm^3. Onion seeds were planted about 1.3 cm deep on March 24, 1972.

From a stock solution of ^{14}C-prynachlor (4.8 $\mu c/\mu$ mole) containing 90 μc in 4.5 ml of ethanol, 30 ml of treatment solution was prepared by dilution with distilled water. Ten ml of solution containing 1.372 mg of ^{14}C-herbicide was applied to the soil surface of each pot providing an application rate equivalent to 4.48 kg/ha.

After herbicide application, the plants were maintained in a growth chamber with a 30°C day (14 hours) and 20°C night (10 hours) for the first 52 days. During this period, each cup received approximately 20 ml of water per day. To develop the plants to maturity, they were transferred to a greenhouse with a night temperature of 21°C and day temperatures ranging from 21 to 32°C. In order to provide adequate room for bulb development, each intact soil mass with plants was transferred to a 6 inch pot. Each pot received approximately 700 ml of water every 2 days until the bulbs reached maturity. Single plants were removed from each cup 11, 17, 24, 82, and 102 days after treatment. Care was taken to retain as many roots as possible on the plant. The roots

were rinsed in distilled water for 30 seconds to remove adhering soil and/or chemical. Plants were separated into root and shoot and immediately frozen with dry ice and acetone. The plants were dried at 45°C and weighed prior to combustion. In the first 4 harvests, the entire plant sample was combusted, whereas in later harvests, samples of 25 to 100 mg from finely ground composites were utilized.

Two plants in each pot were maintained until bulbs were produced of marketable size. These 6 plants were harvested 162 days after treatment and the soil was washed from the roots and bulbs. Each plant was divided into bulb, shoot and root sections and fresh weights were obtained. The tissue was chopped, frozen, and dried as previously described. After obtaining dry weights and grinding the tissue, 100 mg samples were combusted.

Combustion was accomplished with a Nuclear Chicago Model 3151 Oxidizer unit equipped with magnetic stirrers. One liter flasks were purged with oxygen and stoppered with rubber septum cups prior to combustion. Upon cooling of the flasks, 10 ml of ethanol:ethanolamine (2:1 V/V) was injected and they were stirred for 15 minutes. One ml of the CO_2 trapping solution was removed for liquid scintillation counting. A cocktail was prepared by dissolving 4 grams of BBOT (2, 5-bis 5-tert buty-1-benzoxezolyl (2') thiopen) in a liter of toluene. The cocktail counted at 63% efficiency as determined by External Standardization on a Parkard Tricarb Scintillation Spectrometer. All cpm data were converted to dpm.

Uptake and metabolism by 3 week old plants. Onion and proso millet were seeded into 15 by 10 by 7 cm styrofoam boxes containing vermiculite. The seedlings were maintained in a growth chamber with 30°C day (14 hours)

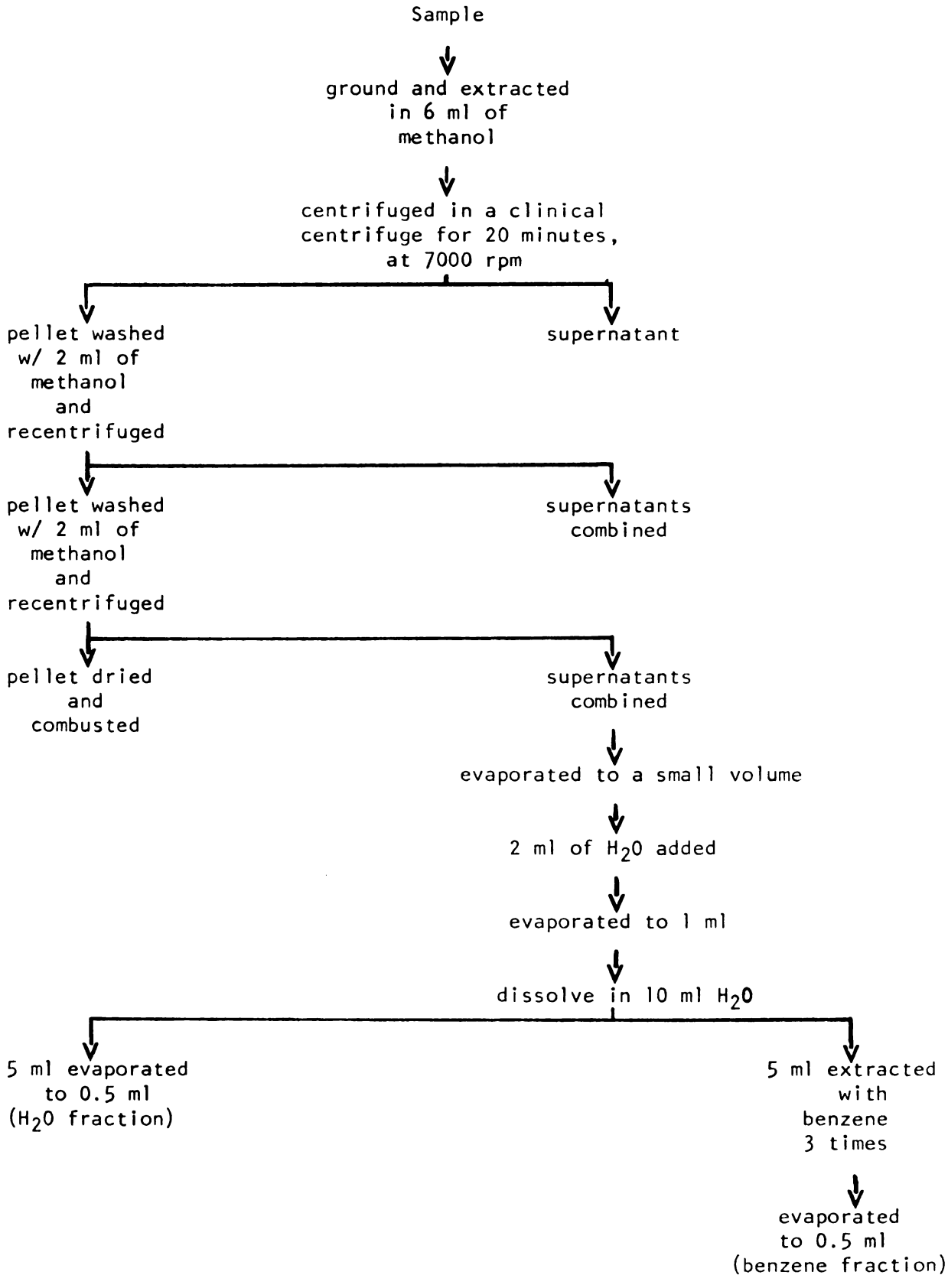
and 20°C night (10 hours) temperatures and watered daily with 100 ml of water. After 2 weeks, plants were transferred to 180 ml plastic containers wrapped in aluminum foil. Half strength Hoaglands solution (14), was used as a growth media and changed every third day during the course of each experiment. A circular sponge which fitted the top of the container was used as a support for the plants. After transplanting, the plants were returned to the growth chamber.

At 3 weeks of growth, each cup was spiked with 0.1 μc ^{14}C-prynachlor in 10 μl of ethanol. Treated plants were returned to growth chamber and harvested 12, 24, 48, and 96 hours after treatment. Harvest, combustion, and counting procedures were similar to those in the previous study.

For metabolism studies another group of plants were treated with 0.4 μc ^{14}C-prynachlor in 40 μl of ethanol. Plants were returned to a growth chamber for 18 hours and then harvested, frozen, and extracted in a 50 ml tissue homogenizer as indicated (Figure 1).

One hundred μl from each prepared extract was streak applied to 250 micron silica gel H thin layer plates. Plates were allowed to develop for 15 cm in a benzene:methanol (97:3 V/V) solvent system. Developed plates were cut into 1 cm sections and each section placed into a vial containing 10 ml of liquid scintillation fluid for counting.

Figure 1. Extraction procedure to remove ^{14}C-prynachlor and metabolites from onion and proso millet seedlings.


Sample
ground and extracted in 6 ml of methanol
centrifuged in a clinical centrifuge for 20 minutes, at 7000 rpm
pellet washed w/ 2 ml of methanol and recentrifuged
supernatant
pellet washed w/ 2 ml of methanol and recentrifuged
supernatants combined
pellet dried and combusted
supernatants combined
evaporated to a small volume
2 ml of H_2O added
evaporated to 1 ml
dissolve in 10 ml H_2O
5 ml evaporated to 0.5 ml (H_2O fraction)
5 ml extracted with benzene 3 times
evaporated to 0.5 ml (benzene fraction)

Results and Discussion

Residue study. At the first harvest (11 days), nearly 10 fold higher concentrations of ^{14}C-compounds were detected in the onion shoots than in the roots (Table 1).

Table 1. Total ^{14}C-residue in onion plants after a preemergence application of prynachlor on a Houghton muck soil.

Days after treatment	Stage of growth	Roots	Shoots	Bulbs
			(dpm/mg)	
11	Flag	1,421	13,808	-
17	Cotyledon	10,605	8,717	-
24	1 Leaf	2,806	2,990	-
82	4 Leaf	4,835	1,031	714
102	5-6 Leaf	413	193	284
162	Mature bulb	.207	.064	.055

This indicates that as the onion cotyledon emerges through the zone of highest herbicide concentration, it is able to absorb the herbicide. Later, as the herbicide moves into the root zone, relatively higher concentrations are detected in the root tissue, a trend that continues to maturity. There is a possibility that some of the ^{14}C-compound may be recycled in the plant. The total amount of ^{14}C-residues per plant increased from the 11 to 82 day sampling date, while the concentration

expressed on a weight basis steadily decreased over the same time period. After 82 days, there was a rapid decrease both in the total amount per plant and in concentration. The concentration detectable in the onion bulb and shoot at harvest was negligible and only about one-fourth that which occurred in the roots. If one assumes that 1 microgram and 1 nanogram of ^{14}C-prynachlor or metabolites decay at a rate of 48,000 dpm and 48 dpm, respectively residues may then be expressed on a parts per billion (ppb) basis. The mature bulbs contained only 1.0 ppb on a dry weight basis and approximately 0.1 ppb expressed on a wet weight basis.

Uptake and metabolism. In 3 week old plants, uptake by onion exceeded that in proso millet (Figure 2). The majority of the activity remained in the roots of both species. Onion roots reached maximum uptake in 24 hours, whereas activity in proso millet roots continued to increase through 96 hours. After 48 hours, transport from root to shoot apparently ceased.

Analysis of the aqueous fraction, showed that both root and shoot of onion rapidly metabolized prynachlor (Figure 3). Over 90% was converted to non-toxic, 2-oic-N-(1-methyl-2-propynyl) acetanilide. Less than 2% of the activity was identified, as parent compound. The benzene fraction contained only 11% of the activity as parent ^{14}C-prynachlor. A distribution of metabolites similar to that found in onion was observed in root and shoot of proso millet.

Vigorously growing onion and proso millet can rapidly absorb and metabolize prynachlor to non-toxic metabolites. This may explain the ineffectiveness of prynachlor on older seedlings.

Figure 2. Uptake of ^{14}C-prynachlor from nutrient culture by 3 week old onion and proso millet seedlings.

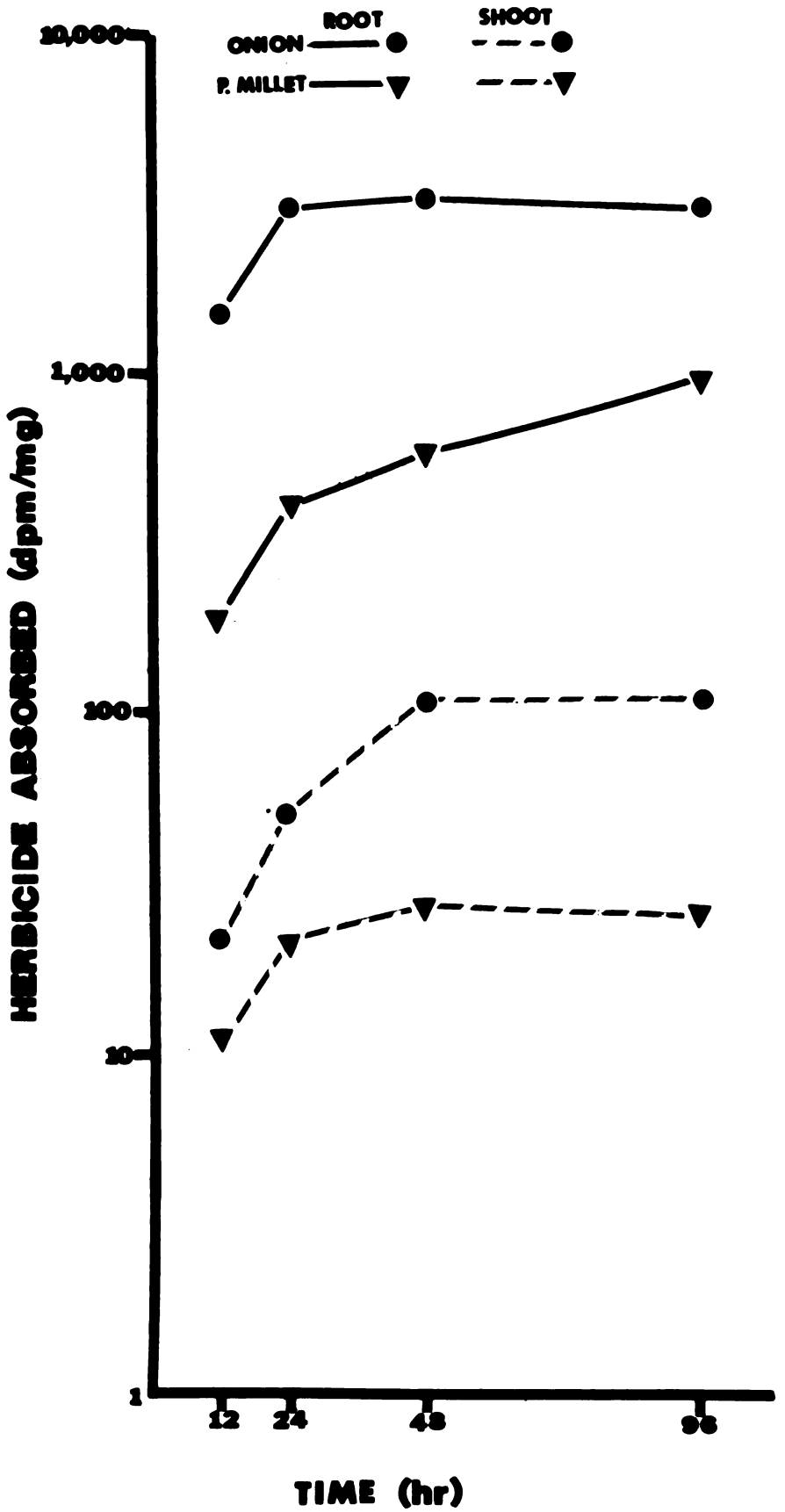
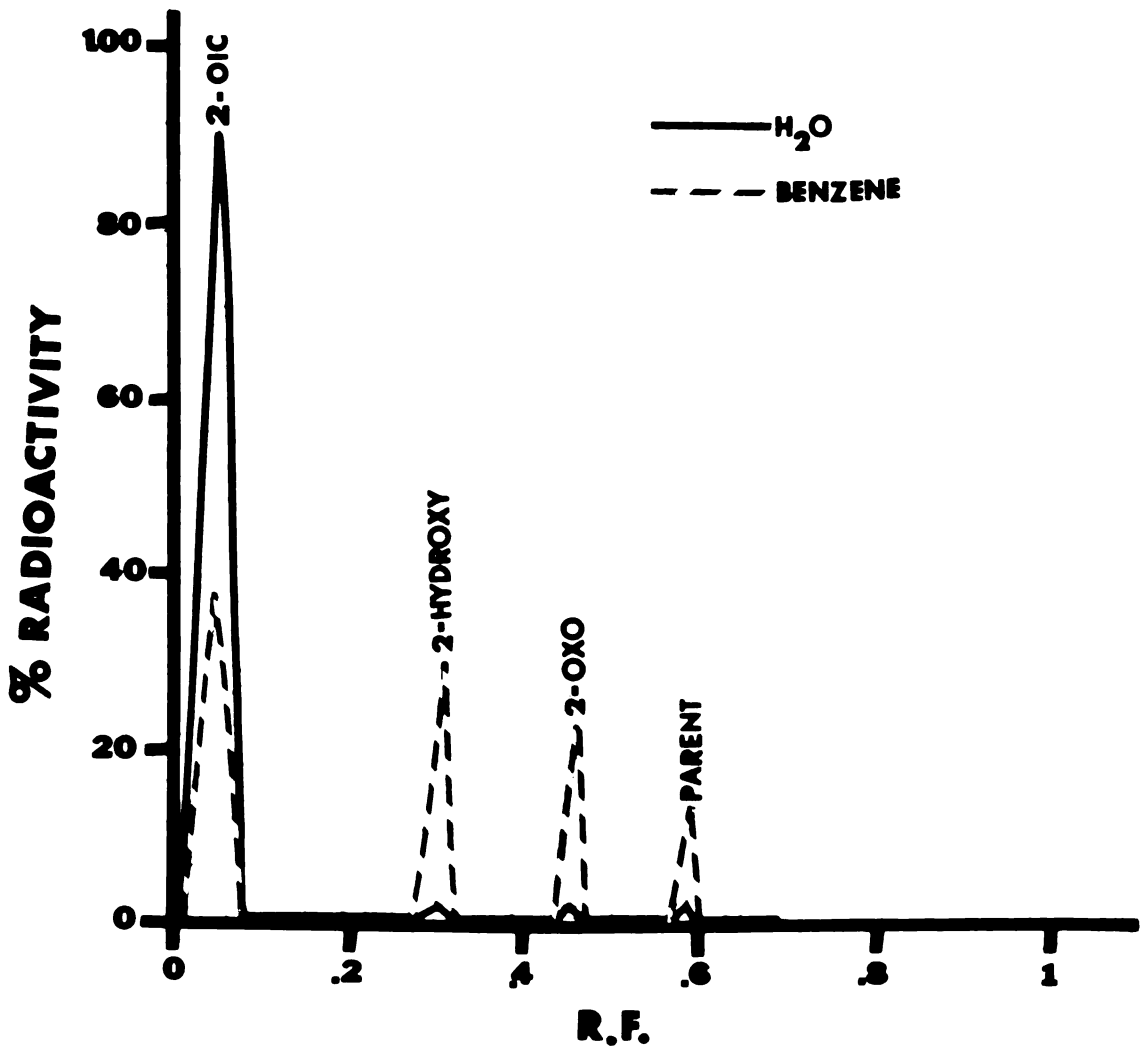

ROOT
SHOOT
ONION
P. MILLET
10,000
1,000
100
10
1
HERBICIDE ABSORBED (dpm/mg)
12
24
48
96
TIME (hr)

Figure 3. Distribution of ^{14}C-prynachlor (parent), 2-oic-N-(1-methyl-2-propynyl) acetanilide, 2-hydroxy-N-(1-methyl-2-propynyl) acetanilide, 2-oxo-N-(1-methyl-2-propynyl) acetanilide in onion and proso millet after 96 hours.


100
80
60
40
20
0
% RADIOACTIVITY
2-OIC
2-HYDROXY
2-OXO
PARENT
H_2O
BENZENE
0
.2
.4
.6
.8
1
R.F.

CHAPTER 5

Summary and Conclusions

The herbicidal activity, uptake, transport and metabolism of prynachlor by onion and proso millet may be summarized as follows:

1. Preemergence application of prynachlor at rates of 4.48 kg/ha or higher effectively controlled many annual grasses. and selected broadleaved weeds on a Houghton muck soil for 6 weeks and did not reduce yield.
2. Preemergence or postemergence applications at the loop, flag, and one leaf stages at rates up to 6.72 kg/ha did not reduce plant weights.
3. On muck soil, onion showed less than 50% reduction in growth at 11.2 kg/ha, but complete kill was achieved at 6.72 kg/ha on mineral soil. This indicates that organic matter imparts some protection.
4. Proso millet, a susceptible species, was killed at 1.12 kg/ha on muck soil and 0.56 kg/ha on mineral soil.
5. Root uptake exceeded shoot uptake in onion and proso millet seedlings when the herbicide was equally available in each zone.
6. The rate of uptake by proso millet root exceeded that of onion roots.
7. Seedlings of proso millet transported a higher percentage of ^{14}C-prynachlor from root to shoot than onion seedlings.

8. Onion seedlings metabolized ^{14}C-prynachlor more rapidly than proso millet seedlings.
9. After treatment on muck soil, onions in the flag stage contained nearly 10 fold higher concentrations of ^{14}C-prynachlor in the shoot than in the root.
10. In 3 week old plants, uptake by onion exceeded proso millet and the majority of the activity remained in the roots of both species.
11. Both proso millet and onion at 3 weeks of age, metabolized over 90% of parent prynachlor to non-toxic 2-oic-N-(1-methyl-2-propynyl) acetanilide after 18 hours of uptake.
12. Mature onion bulbs contained about 1 part per billion ^{14}C-prynachlor or metabolites after a single preemergence application of 4.48 kg/ha.

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