BENTAZON SELECTIVITY AND METABOLISM

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THESIS

ABSTRACT

BENTAZON SELECTIVITY AND METABOLISM

By

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The responses of soybean (*Glycine max* (L.) Merr.), navy bean (*Phaseolus vulgaris* L.), cocklebur (*Xanthium pennsylvanicum* Wallr.) and black nightshade (*Solanum nigrum* L.) to postemergence applications of bentazon (3-isopropyl-1*H*-2,1,3 benzothiadiazin-(4) 3*H*-one 2,2-dioxide) at the rate of 1.12 kg/ha, were evaluated in greenhouse studies.

The basis for selectivity of bentazon allowing navy bean tolerance, cocklebur susceptibility and black nightshade moderate susceptibility was evaluated by studying spray retention, translocation and metabolism. Translocation and metabolism were also examined in tolerant soybean.

The trifoliate leaves of navy bean retained less bentazon than the unifoliate leaves of navy bean and the leaves of seedling cocklebur and black nightshade. The ^{14}C-labelled compounds from ^{14}C-bentazon applied to the foliage of black nightshade was translocated throughout the entire plant. In navy bean, the ^{14}C-labelled compounds

were localized in the treated area of the first trifoliate leaflet with acropetal movement. However, acropetal and basipetal movement were observed throughout the unifoliate navy bean and trifoliate soybean seedlings. In cockelbur, the ^{14}C -labelled compounds diffused throughout the entire treated leaf only.

There were differences among species in the rate of metabolism and the ^{14}C-metabolites formed from ^{14}C-bentazon 1 and 5 days after treatment. Metabolism of bentazon was most rapid in the trifoliate leaves of navy bean.

Translocation and metabolism studies were also conducted in navy bean seedlings with their first fully expanded trifoliate leaf, following prior treatments with EPTC (S-ethyl dipropylthiocarbamate), chloramben (3-amino-2,5-dichlorobenzoic acid) and trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine). Acropetal and basipetal translocation of ^{14}C-labelled compounds occurred in EPTC pretreated navy beans. Although prior herbicide treatments did not decrease the rate of metabolism of the kinds of metabolites found, the percentage of metabolites varied among treatments.

In soybean, four apparent ^{14}C-conjugates were found in plants allowed to grow until full maturity.

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INTRODUCTION

Bentazon (3-isopropyl-1*H*-2,1,3 benzothiadiazin-(4)3*H*-one 2,2-dioxide) has shown considerable promise as a post-emergence herbicide for selective control of many broad-leaved weeds in soybeans (*Glycine max* (L.) Merr.) (4, 19). Weeds selectively controlled include cocklebur (*Xanthium* pennsylvanicum Wallr.), wild mustard (*Brassica kaber* D.C.), common ragweed (*Abutilon theophrasti* Medic.).

In Michigan, navy bean (*Phaseolus vulgaris* L.) acreage approximates the soybean acreage with similar weed problems. Several preplant incorporated and preemergence herbicides, currently being used for the two crops, are the same. However, in many instances, they are not effective in both navy bean and soybean. Bentazon has also shown promise as a postemergence herbicide for the control of broadleaved weeds in navy beans (20). However, effective total weed control in both navy bean and soybean requires bentazon combinations with preplant incorporated or preemergence herbicides.

The purpose of this research was to determine the basis for the selectivity of bentazon between navy bean and some weed species, to determine the effect of prior herbicide treatments on navy bean tolerance to bentazon, and to compare metabolism of bentazon in navy bean with soybean.

Chapter 1

LITERATURE REVIEW

Weed Problems in Navy Bean and Soybean

In the United States, weeds in soybean (*Glycine max* (L.) Merr.) have been found to reduce the potential value of the crop almost 17 percent per year (12). McWhorter and Hartwig (9) found that common cocklebur (*Xanthium pennsylvanicum* Wallr.) alone can reduce the average yields in certain soybean varieties by as much as 75%.

Hicks et al. (9) have shown that light penetration within the soybean canopy influenced soybean yields. Reduced penetration of light into the canopy due to a heavy weed infestation, reduced soybean yields.

Weeds can also increase lodging of soybean plants. Weber and Fehr (18) reported yield losses of 32 kg/ha due to this cause.

Navy bean (*Phaseolus vulgaris* L.) fields, with a heavy infestation of cocklebur usually show reduced vigor, growth and yield, which is generally attributable to the more rapid and larger growth of cocklebur. Weeds may also reduce the quality of navy bean seed because of the nature of the harvesting method. In Michigan, harvesting navy beans together with the purple black nightshade (*Solanum nigrum* L.)

berries causes discoloration to the navy bean seed. This reduction in quality has caused considerable economic losses to Michigan navy bean growers.

Postemergence Herbicides for Weed Control in Soybean and Navy Bean

Preemergence herbicides frequently give less than the desired level of weed control in both soybean and navy bean. This has generated considerable interest in compounds that may be applied in a postemergence manner. Herbicides applied in this manner to soybean include chloroxuron (3-[*p*-(*p*-chlorophenoxy) phenyl]-1,1-dimethyl urea), 2,4-DB (2, 4-dichlorophenoxy butyric acid), and bromoxynil (3,5-dibromo-4-hydroxybenzonitrile). Bentazon (3-isopropyl-1*H*-2,1,3 benzothiadiazin-(4)3*H*-one 2,2-dioxide) currently has an experimental label for this use.

Postemergence applications of chloroxuron to soybean, at rates of 0.84, 1.12, 1.68, and 3.36 kg/ha, injured and initially stunted all plants (2, 5, 11). The leaves exhibited a burned appearance with some subsequent defoliation. Delayed maturity, reduced stands and yields were also reported (2, 5, 11). When these effects were observed, the soybean had only their unifoliate leaves. Applications to soybeans having their first, second and third trifoliate leaves seldom delayed maturity or reduced yields. Parachetti et al. (1) found that postemergence applications of chloroxuron to soybeans did not significantly reduce yields if applied after a preemergence soybean herbicide, provided the soybeans had at least one fully expanded trifoliate leaf.

Baldwin and Frans (5) controlled cocklebur with chloroxuron only if the plant was in its cotyledonary stage of growth, and a high (3.36 kg/ha) rate was applied. Gossett et al. (8) obtained excellent control of cocklebur with chloroxuron at rates of 0.56 and 1.12 kg/ha if a surfactant (dodecyl ether polyethylene glycol) was used and the cocklebur seedlings were under 8 cm in height.

The selective action of chloroxuron on soybean (tolerant species) and tall morning glory (Ipomea purpurea (L.) Roth) (susceptible species) was examined by Feeny et al. (6). Selectivity of postemergence applications was based on differential foliar absorption and greater retention of chloroxuron by the susceptible species. Tall morning glory contained eight times more radioactivity than soybean one-week-old seedlings. Chloroplasts from tall morning glory initially retained two times more chloroxuron than did chloroplasts from soybean after foliar treatment. Differential metabolism was not considered as a basis of selectivity between these two species.

When chloroxuron was root-applied, soybean accumulated more chloroxuron in the root than did tall morning glory. However, the soybean roots appeared to be effective barriers to acropetal transport as tall morning glory allowed chloroxuron to acculuate in the foliage causing phytotoxicity.

Chloroxuron is a photosynthetic inhibitor, so accumulation in the photosynthetic tissue is critical.

Postemergence applications of 2,4-DB at the rate of 0.44 kg/ha resulted in more injury to soybean than chloroxuron applied at 3.36 kg/ha (8, 16). 2,4-DB also reduced soybean yields more than chloroxuron. However, excellent control of cocklebur has been obtained with 2,4-DB with rates as low as 0.44 kg/ha (8).

Wathana et al. (15) compared the uptake and translocation of 2,4-DB in soybean and cocklebur. The penetration rate of 2,4-DB was faster in cocklebur than in soybean after one day. In soybean, only trace quantities of 2,4-DB were translocated from the treated leaf to the other plant parts. In cockelbur, there was considerably greater movement of the herbicide into the meristematic tissues of the plant.

Bromoxynil has also been shown to cause early injury and delayed maturity to soybeans when applied at rates of 0.07, 0.14, 0.196 and 0.252 kg/ha (3). However, excellent cocklebur control was obtained if the high rates of bromoxynil were used. Wax et al. (17) also found some yield reduction from bromoxynil treated soybeans at rates of 0.3 and 0.4 kg/ha.

Van Amsberg et al.[1] first reported the use of bentazon as a selective postemergence herbicide for the control of

[1] Von Amsberg, H., J.P. Pearson, J.W. Daniel, A.L. Weishar, C.W. Carter, M.A. Veenstra, J.T. Thompson, and B. Wuerzer. 1971. Effects of 3-isopropyl-1*H*-2,1,3-benzothiadiazin-(4) 3*H*-one 2,2 dioxide (Bas-3512-H) on Soybeans and Broadleaved Weeds at Various Stages of Growth. Weed Sci. Soc. Amer. Abstr. No. 106.

broadleaved weeds in soybeans. They reported that soybeans with unifoliate, and first through fourth trifoliate leaves were tolerant to applications of bentazon at rates ranging from 0.84 to 2.24 kg/ha. Common cocklebur was susceptible to bentazon at all rates used. Cocklebur was found to be susceptible up to and occasionally including the flowering stage. Weber et al. (19) found minimal injury to soybean, and excellent control of cocklebur with bentazon at the rate of 1.12 kg/ha. Wax et al. (17) found soybean to be tolerant to bentazon applications of 3.36 kg/ha at the unifoliate and first and second trifoliate stages of growth. Soybean was also found to be more tolerant to applications of bentazon than bromoxynil, chloroxuron and 2,4-DB. When applications of these herbicides were made on soybean cultivars introduced from Japan, extensive injury resulted. The herbicide causing the least amount of injury to these cultivars was chloroxuron (17). Anderson et al. (13) found reductions in yield from bentazon applications under high soil moisture conditions. Soybeans grown in soil with moisture levels not exceeding field capacity showed no injury to postemergence applications of bentazon.

Penner (12) related selectivity of bentazon between tolerant soybean and susceptible Canada thistle (*Circium arvense* L.) to differences in leaf retention and the rate of bentazon metabolism. Less bentazon was retained on the leaves of soybean than in Canada thistle. After one day, 25 to 35 percent bentazon remained unmetabolized in Canada thistle, whereas only 16 percent of the bentazon remained unmetabolized in soybean.

Bentazon has been shown to exhibit a strong movement toward the anode in an electrical field in water or a neutral solution (1). It leached quite readily from the soils and was not adsorbed to any of the soils tested thus far.

Bentazon adsorbed readily to an anion exchange resin, but did not adsorb to a cation exchange resin at a pH of 7. When the pH of both resins was adjusted to 2 or 12, a small amount of bentazon did adsorb to the cation exchange resin, while less was adsorbed to the anion exchange resin than that found at a neutral pH. These results indicate that bentazon exhibits strong anionic properties when placed in neutral solutions or soils with a pH range of 5 to 7. In nonflooded soils, the absorption of bentazon from the soil does not seem to be a factor affecting its action.

Currently, there are no postemergence herbicides for navy bean, kidney bean, pinto bean, etc. (Phaseolus vulgaris L.), that control weeds without causing extensive injury and yield reduction to these beans.[2] Bentazon, which shows promise as a postemergence herbicide for soybeans, also appears promising as a postemergence herbicide for the control of certain broadleaf weeds in other beans. Preplant incorporated or preemergence herbicides must be used in

[2]Personal Communication. Donald Penner, Michigan State University, East Lansing, Michigan.

combination with bentazon if effective, overall weed control is to be achieved.[3] Fenster and Wicks (7) have shown good to excellent weed control in Great Northern "No. 59" field beans with bentazon at the rate of 0.84 kg/ha in combination with preemergence applications of alachlor (2-chloro-2',6'-diethyl -*N*-(methoxymethyl) acetanilide) at the rate of 2.24 kg/ha and preplant incorporated EPTC (*S*-ethyl dipropylthiocarbamate) at the rate of 1.68 kg/ha. In some cases slight injury did occur, but there was no significant yield reduction if compared to the hand weeded check.

In Michigan, navy beans are a major crop. The use of bentazon in combination with preplant incorporated or preemergence herbicides for weed control is also of interest. Bentazon applied alone at the rate of 1.68 kg/ha controlled 88 percent of the black nightshade. In some cases, complete kill was not obtained and regrowth of black nightshade did occur (20). Bentazon applications at the rate of 1.12 kg/ha in combination with preplant incorporated treatments, gave excellent control of all broadleaved weeds present.

Bentazon applications at rates of 0.84, 1.12, and 1.68 kg/ha to navy beans before the first trifoliate leaf was fully expanded, resulted in severe injury accompanied by stand reduction.[4] However, bentazon applications at the same rates to navy beans with the first and second trifoliate leaves fully expanded caused only slight injury, and the

[3]Personal Communication. Donald Wyse, Michigan State University, East Lansing, Michigan.
[4]*Ibid*.

yields did not differ significantly from the hand weeded check.[5]

[5] *Ibid*.

Chapter 2

BENTAZON TRANSLOCATION AND METABOLISM IN SOYBEAN AND NAVY BEAN

Abstract

Bentazon (3-isopropyl-1*H* 2,1,3 benzothiadiazin-(4)3*H*-one 2,2-dioxide) shows promise as a selective postemergence herbicide for the control of weeds in soybean (*Glycine max* (L.) Merr.) and navy bean (*Phaseolus vulgaris* L.).

Following foliar ^{14}C-bentazon application, the movement of ^{14}C was primarily acropetal in both species. However, basipetal movement was also observed.

Metabolism was more rapid in the trifoliate navy bean leaf than in the unifoliate navy bean leaf and the trifoliate soybean leaf. Prior herbicide treatments did not decrease metabolism of bentazon in navy beans. The metabolites in navy bean and soybean appeared similar. Four apparent ^{14}C-conjugates were isolated from soybean plants harvested at full maturity.

Introduction

Current preplant and preemergence applications of herbicides available for weed control in soybean and navy bean do not control the full range of problem weeds. Broadleaf weed infestations, such as cocklebur (Xanthium pennsylvanicum Wallr.), have increased due to the decrease in competition from other weeds controlled by preplant and preemergence applications of herbicides (4, 6).

Postemergence applications of 2,4-DB (2,4,-dichlorophenoxy butyric acid), chloroxuron (3-[p-(p-chlorophenoxy) phenyl]-1,1-dimethyl urea) and bromoxynil (3,5-dibromo-r-hydroxybenzonitrile) to soybean control many broadleaf weeds, such as cocklebur, but may also cause extensive injury to soybean (1, 2, 4, 6, 7, 9, 10). Bentazon, used as a postemergence herbicide for weed control in soybean, has shown excellent control of cocklebur and other problem weeds with minimum injury to soybean (3, 11). Bentazon also shows promise as a postemergence herbicide for weed control in navy bean[1] (5). However, bentazon injury to navy bean plants having only unifoliate leaves has been observed in the field.

Our objectives were to determine the movement of ^{14}C from ^{14}C-bentazon in soybean and navy bean, to examine differences in translocation of ^{14}C between unifoliate and first trifoliate leaves of navy bean, to determine the influence of herbicide pretreatments on bentazon translocation and metabolism, and to compare bentazon metabolism in soybean and navy bean.

[1]Wyse, D.L., W.F. Meggitt and R.C. Bond. 1973. New herbicides for navy beans. Research Report, N. Cent. Weed Contr. Conf. 28:76-79.

Materials and Methods

"Hark" soybean and "Sanilac" navy bean were grown in Conover sandy loam soil in the greenhouse with supplemental fluorescent lighting. For the study on the influence of herbicide pretreatments, EPTC (S-ethyl diporpylthiocarbamate) at 3.36 kg/ha, trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-P-toluidene) 0.56 kg/ha were applied preplant incorporated and chloramben (3-amino-2,5-dichlorobenzoic acid) at 2.24 kg/ha was applied in a preemergence manner.

To study the translocation of ^{14}C-bentazon in navy bean and soybean seedlings, a 5μl droplet containing 0.2μCi of ^{14}C-bentazon was placed at the base of the unifoliate leaf or at the base of the center leaflet of the first trifoliate leaf. The bentazon had a specific activity of 2.97 mCi/m-mole and was labelled in the number 10 position. Plants were harvested 1 and 5 days following treatment, freeze dried, mounted and radioautographed.

For the metabolism study, plants were treated with ^{14}C-bentazon in the same manner as in the translocation experiment. The leaf apecies above the site of ^{14}C-bentazon application were harvested, homogenized in absolute methanol, and the homogenate filtered through Whatman number 1 filter paper under vacuum. The methanol-insoluble portion was combusted under O_2 by the method of Wang and Willis (8), and the radioactivity determined by liquid scintillation spectrometry. The methanol-soluble portion was evaporated to dryness *in vacuo*, resuspended in 15 ml methanol:water (1:2; v/v) and partitioned against 15 ml benzene and ethyl acetate three times in 5 ml fractions. All of the samples were then evaporated to dryness *in vacuo* and resuspended in 1 ml of their respective solvents. One-hundred µl of each fraction was then radioassayed and 400 µl were spotted on 250 micron thick silica gel F-254 thin layer chromatography plates. The plates were developed in a solvent system of chloroform:methanol (7:3; v/v). After development to a height of 15 cm, the plates were radioautographed. The ^{14}C-labelled spots on the plate were removed and radioassayed by liquid scintillation spectrometry.

Methanol-soluble extracts from soybean, allowed to grow until they reached full maturity, were obtained from the BASF Wyandotte Corporation. The nature of the ^{14}C-metabolites was determined by gel filtration or molecular sieve chromatography, with subsequent spotting on thin layer plates as in the metabolism study. The methanol-soluble extracts were concentrated from 10 ml to 1 ml. Five-tenths ml were then placed on a sephadex G-15 gel column 15 cm high and 1 cm in diameter. The columns were eluted with water at a flow rate of 0.075 ml/min. One ml fractions were collected and 200 µl from each fraction radioassayed by liquid scintillation spectrometry. The fractions showing the greatest quantities of radioactivity were then spotted on thin layer plates and developed as previously stated.

All data presented in the tables are the means of two experiments with three replications per experiment. Figures are representative of two or more experiments.

Results and Discussion

Foliar applications of ^{14}C-bentazon to the unifoliate leaves of navy bean seedlings resulted in acropetal and basipetal trnaslocation of the ^{14}C from the site of application (Figure 1). After 5 days the extent of movement was similar to that found after 1 day. Foliar applications made to the center leaflet of the first trifoliate leaf of navy bean resulted in acropetal but no basipetal movement of ^{14}C from the site of application after 1 or 5 days (Figure 2). This could be a possible explanation of why navy bean plants having trifoliate leaves are more tolerant to foliar applications of bentazon than plants having only unifoliate leaves.

Navy bean grown in EPTC-treated soil showed more acropetal movement of ^{14}C in the treated trifoliate leaflet 1 day after treatment than was observed in plants not receiving the herbicide pretreatment (Figures 2 and 3). However, the EPTC treatment did not alter navy bean tolerance to foliar applications of bentazon. The chloramben and trifluralin treatments did not affect ^{14}C movement in navy bean.

In soybean, movement of ^{14}C from foliar applications of ^{14}C-bentazon was primarily acropetal accompanied by some basipetal movement (Figure 4). After 5 days, both acropetal and basipetal movement was more extensive (Figure 4).

The same number of metabolites were found in the unifoliate leaf apex as in the center trifoliate leaflet apex of navy bean (Tables 1 and 2). They were probably the same, since the R_f values were very similar from the leaf apices of the two species. However, the percent of ^{14}C remaining as unmetabolized bentazon was significantly higher in the unifoliate navy bean leaf apex than in the center trifoliate leaflet apex after one day (Table 3). This offers another explanation of the observed differences in tolerance of navy bean at the two stages of growth to foliar applications of bentazon.

Prior herbicide treatments did not significantly decrease the rate of ^{14}C-bentazon metabolism after 1 day in navy bean compared to the control (Table 4). Navy bean growth on EPTC-treated soil showed a significantly increased rate of bentazon metabolism in the center trifoliate leaflet apex compared to the control. This higher rate of bentazon metabolism may counterbalance the increased translocation caused by the EPTC treatments or be an explanation for it. After 5 days, the percent of metabolized bentazon did not differ significantly from that found after 1 day in the center trifoliate leaflet apices of navy bean with and without prior herbicide treatments (Table 5).

Metabolism of ^{14}C-bentazon on soybean and navy bean appeared to be similar in the number of metabolites found and their characteristics. However, most of the R_f values of the ^{14}C-metabolites from soybean were slightly different than those from navy bean (Tables 1 and 6). The percent of unmetabolized bentazon 1 day after ^{14}C-bentazon application was much higher in the center trifoliate leaflet apex of soybean than navy bean (Table 7). Five days after treatment there was a significant reduction in the percent of unmetabolized bentazon in soybean. Because of the slow initial rate of bentazon metabolism in soybean, and the movement of ^{14}C throughout the plant, other factors may also play a role in the observed tolerance of soybean to bentazon.

Methanol-soluble extracts obtained from ^{14}C-bentazon treated soybeans harvested at maturity revealed three peaks of radioactivity when subjected to molecular sieve chromatography (Figure 5). The metabolites appeared to be ^{14}C-bentazon conjugates as eluted from the column between standards with known molecular weights of approximately 698 and 269 (Figure 5). The predominate ^{14}C-metabolite was resolved into two bands of radioactivity by TLC indicative of two metabolites present in this peak. Thus, four apparent ^{14}C-bentazon conjugated metabolites were isolated from ^{14}C-bentazon-treated soybean harvested at maturity.

Metabolism and translocation of bentazon appear to be major factors in the greater tolerance of navy bean, having trifoliate leaves when bentazon was applied, compared to those having only unifoliate leaves. Prior herbicide treatments did not significantly decrease metabolism of bentazon in trifoliate leaflet apecies of navy bean.

Trifoliate leaves of soybean translocated more ^{14}C and metabolized a smaller percentage of bentazon than trifoliate leaves of navy bean, indicating the possible role of other factors contributing to the tolerance of soybean to foliar applications of bentazon.

Figure 1. Translocation of ^{14}C-bentazon in navy bean. The treated plants (A) and the corresponding radioautograph (B) show navy bean with only unifoliate leaves harvested 1 day after foliar application. The treated plants (C) and corresponding radioautograph (D) show navy bean at the same stage of growth harvested 5 days after foliar application.

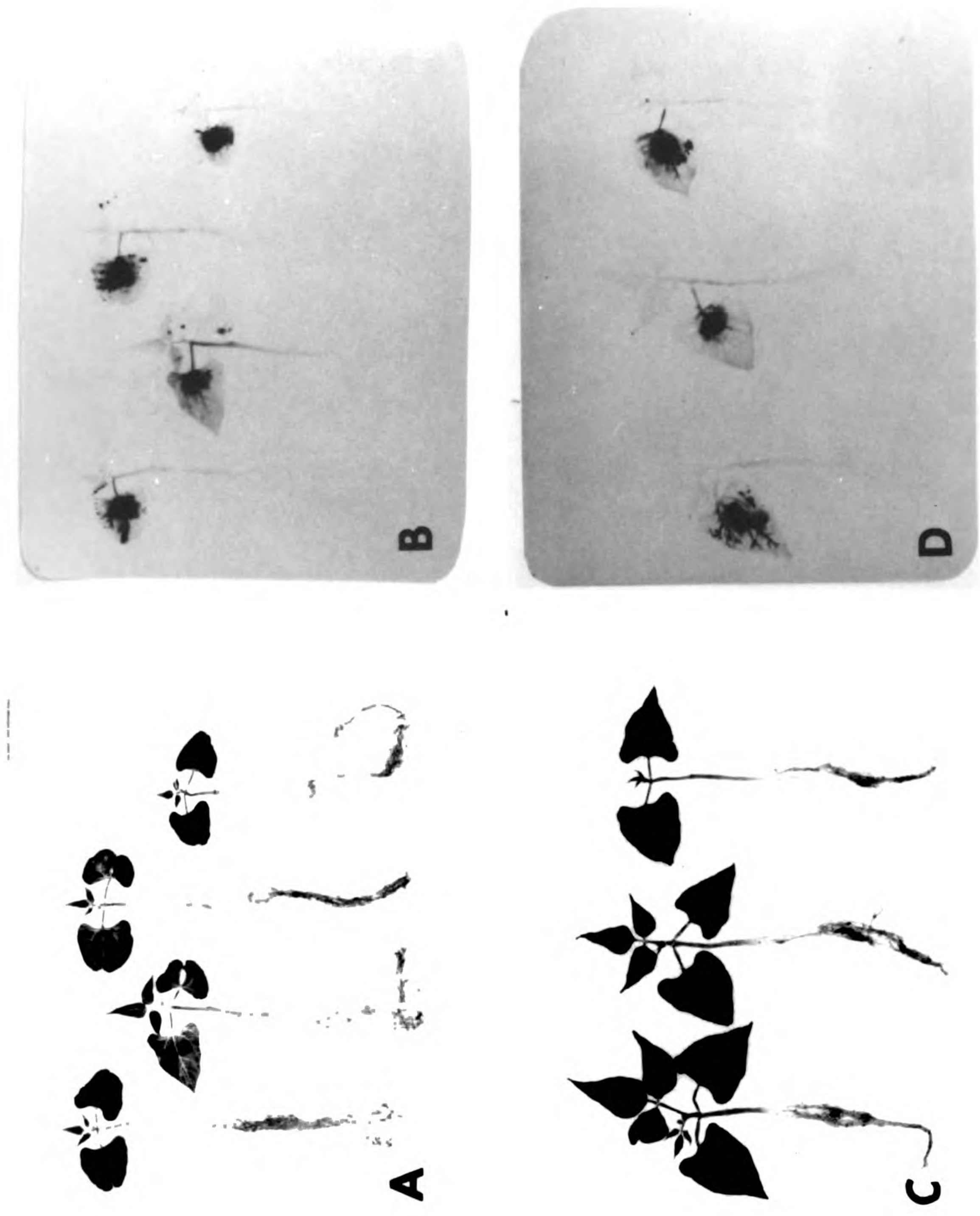
B
D
A
C

Figure 2. Translocation of ^{14}C-bentazon in navy bean. The treated plants (A) and corresponding radioautograph (B) show navy bean having trifoliate leaves, harvested 1 day after foliar application. The treated plants (C) and corresponding radioautograph (D) show navy bean at the same stage of growth harvested 5 days after foliar application.

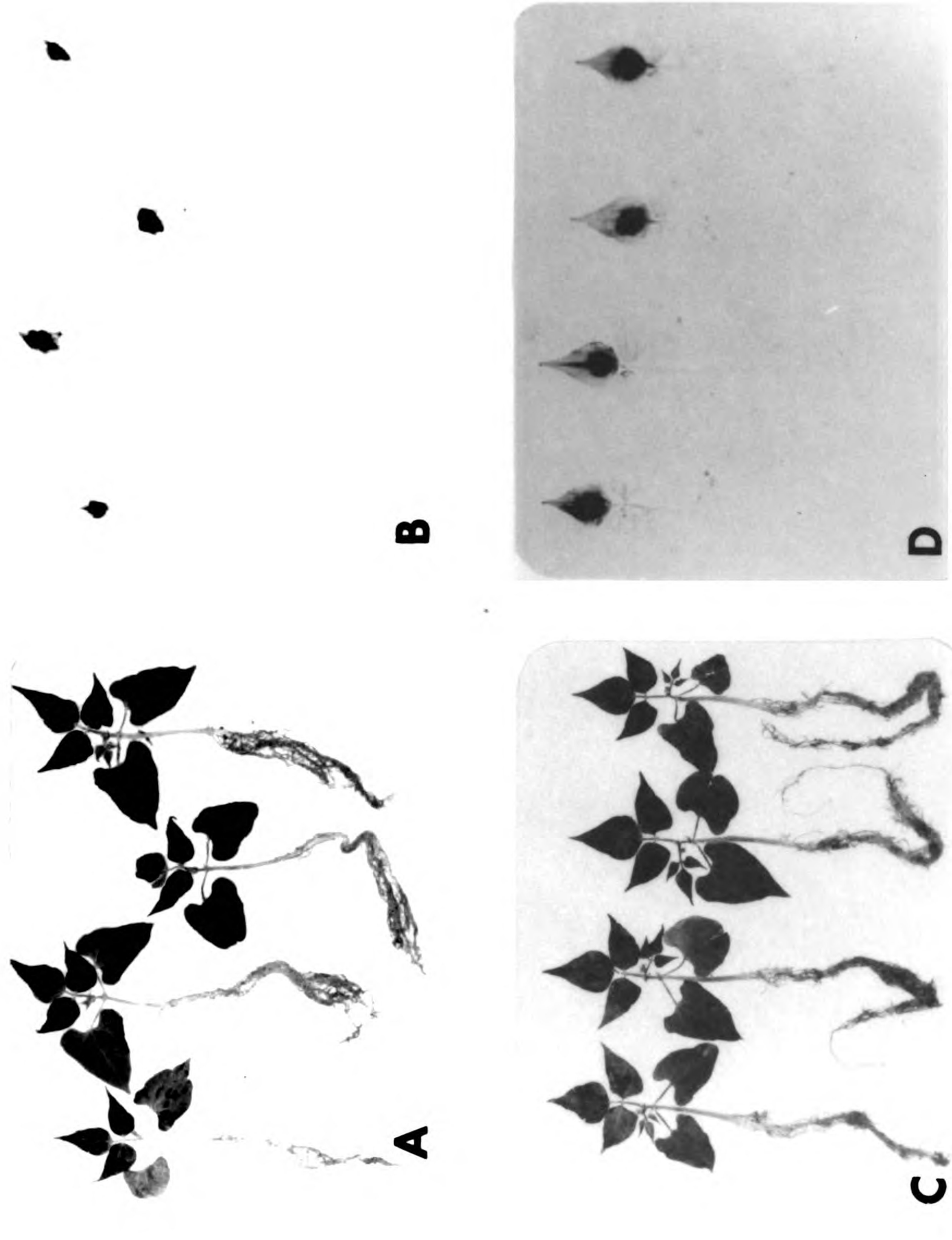
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Figure 3. Translocation of ^{14}C-bentazon in navy bean with a prior herbicide treatment of EPTC. The treated plants (A) and corresponding radioautograph (B) show navy bean having trifoliate leaves harvested 1 day after foliar application. The treated plants (C) and corresponding radioautograph (D) show navy bean at the same growth stage harvested 5 days after foliar application.

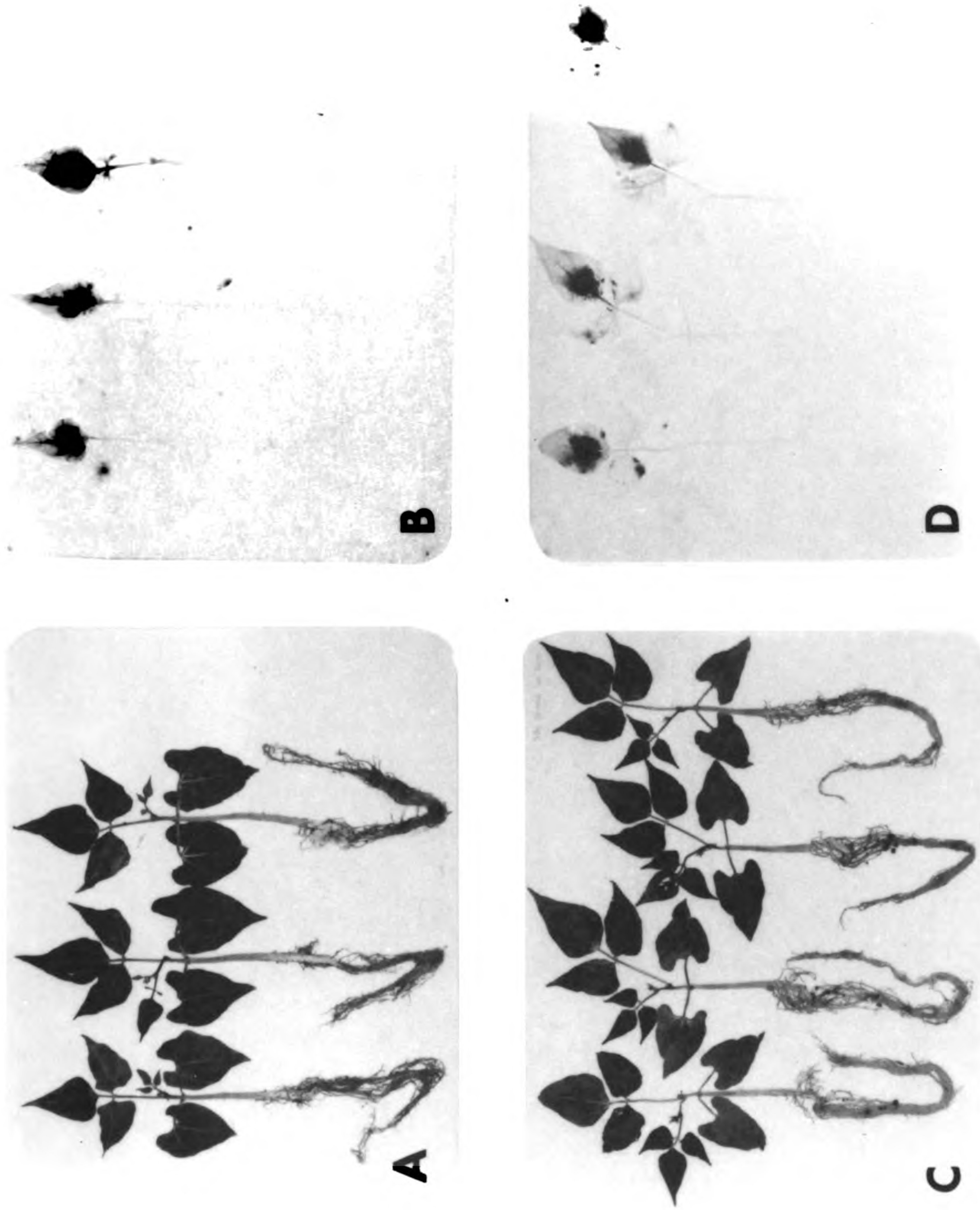
A
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Figure 4. Translocation of ^{14}C-bentazon in soybean. Treated plants (A) and corresponding radioautograph (B) show soybean having trifoliate leaves harvested 1 day after foliar application. Treated plants (C) and corresponding radioautograph (D) show soybean at the same stage of growth harvested 5 days after foliar application.

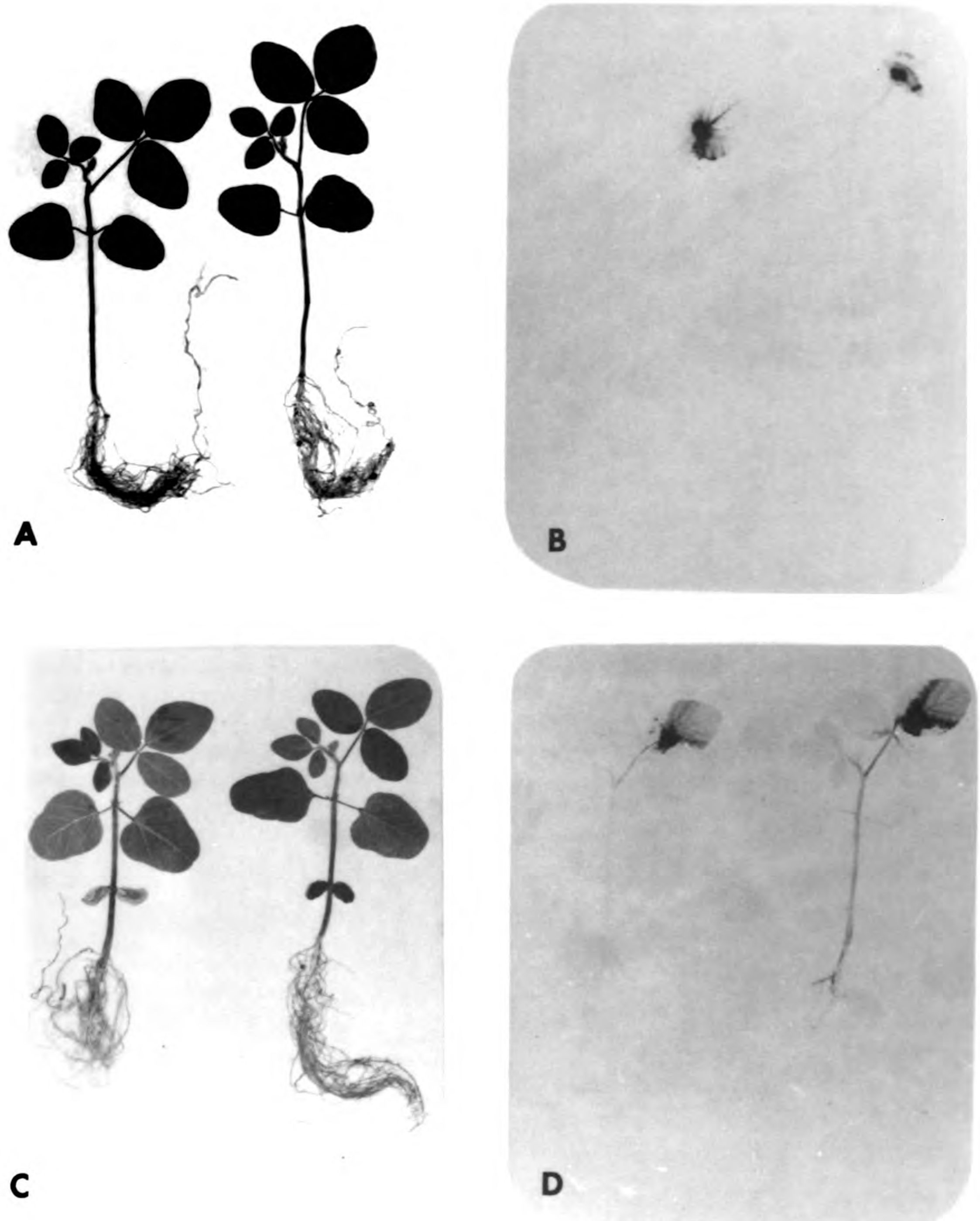
A
B
C
D

Figure 5. Graph showing the characteristics of the ^{14}C-metabolites obtained from the methanol-soluble extract of ^{14}C-bentazon treated soybeans allowed to grow until maturity as determined by molecular sieve chromatography. Peaks B, C, and D show the fractions where the most ^{14}C-metabolites were eluted from the column whose R_f values correspond to those of B, C and D of Table 1.

PLOT OF COLOR INTENSITY OF STANDARDS AND DPM OF METABOLITES OF BENTAZON VS FRACTION NO.

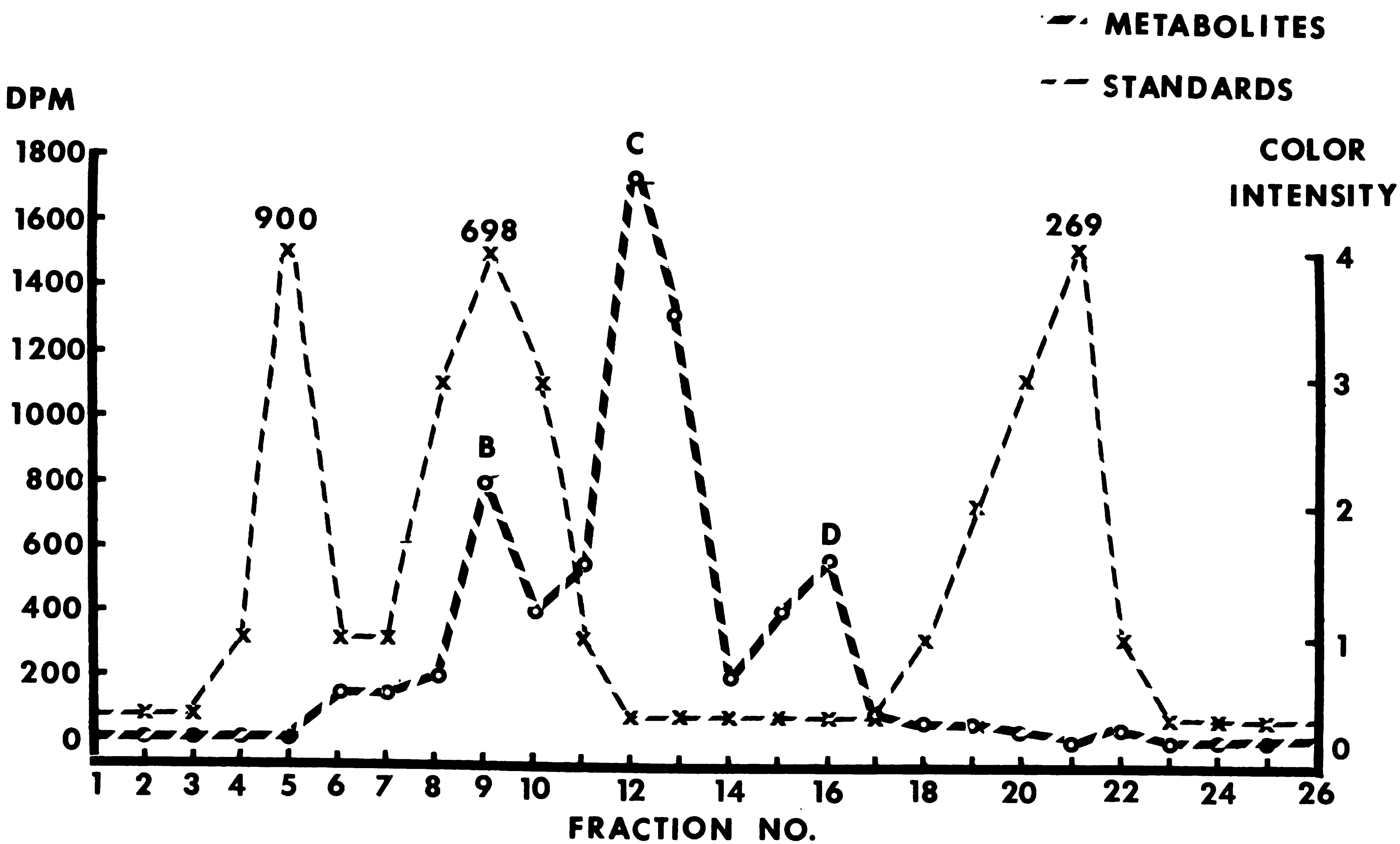

Table 1. R_f values of ^{14}C-bentazon metabolites from the center leaflet of the first trifoliate navy bean leaf.

Metabolites	R_f
Ethyl acetate-soluble	.18
Water-methanol-soluble A	.00
Water-methanol-soluble B	.12
Water-methanol-soluble C	.19
Water-methanol-soluble D	.26
^{14}C-Bentazon	.68

Table 2. Metabolism of ^{14}C-bentazon in the unifoliate leaf apex of navy bean, 1 and 5 days after treatment.[ab]

Treatment duration	Ethyl acetate-soluble	Methanol-soluble				Methanol-insoluble	^{14}C-bentazon
		A	B	C	D		
(days)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	0.6 a	11.1 a	11.0 b	18.6 a	9.6 a	21.7 a	28.8 b
5	0.0 b	17.8 b	5.1 a	21.3 a	9.2 a	38.0 b	8.6 a

[a]Means within columns with the same letters are not significantly different at the 5% level. Percent values were transformed to the arcsine for statistical analysis.

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

Table 3. Comparison of unmetabolized ^{14}C-bentazon between the navy bean unifoliate leaf apex with time and the center navy bean trifoliate leaflet.[a]

Treatment site	Treatment duration (days)	Unmetabolized ^{14}C-bentazon (%)
Trifoliate	1	12.0 a
Unifoliate	1	28.8 b
Unifoliate	5	8.6 a

[a]Means with the same letters are not significantly different at the 5% level. Percent values were transformed to the arcsine for statistical analysis.

Table 4. Metabolism of ^{14}C-bentazon in the center navy bean trifoliate leaflet apex 1 day after ^{14}C-bentazon application following various herbicide treatments.[a,b]

Herbicide treatments	Rate	Ethyl acetate-soluble	Water-methanol-soluble				Methanol-insoluble	^{14}C-bentazon
			A	B	C	D		
	(lb/A)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Control	-	0.7 a	15.0 a	20.5 c	21.0 a	8.3 a	22.5 ab	12.0 bc
EPTC	3.0	1.4 b	16.0 a	20.4 c	21.4 a	8.0 a	27.1 b	5.7 a
Chloramben	2.0	1.2 b	15.0 a	14.1 b	21.6 a	14.0 b	21.6 a	12.5 c
Trifluralin	0.5	0.8 a	15.9 a	10.8 a	26.6 b	8.1 a	27.0 b	10.7 b

[a]Means within columns with the same letters are not significantly different at the 5% level by Duncan's multiple range test. Percent values were transformed to the arcsine for statistical analysis.

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

Table 5. The effect of previous herbicide treatments on the change in percent of unmetabolized ^{14}C-bentazon in center navy bean trifoliate leaflet, 1 and 5 days after ^{14}C-bentazon application.[a]

Treatment duration	Herbicide treatment			
	Control	EPTC	Chloramben	Trifluralin
(days)	(%)	(%)	(%)	(%)
1	12.0 a	5.7 a	12.5 a	10.7 a
5	9.4 a	4.1 a	8.0 a	8.0 a

[a]Means within columns with the same letters are not significantly different at the 5% level. Percent values were transformed to the arcsine for statistical analysis.

Table 6. R_f values of the ^{14}C-bentazon metabolites from the center leaflet of the first trifoliate soybean leaf.

Metabolites	R_f
Ethyl acetate-soluble	.33
Water-methanol-soluble A	.00
Water-methanol-soluble B	.12
Water-methanol-soluble C	.31
Water-methanol-soluble D	.40
^{14}C-bentazon	.67

Table 7. Metabolism of ^{14}C-bentazon in the center leaflet of the first trifoliate soybean leaf, 1 and 5 days after treatment.[ab]

Treatment duration	Ethyl acetate-soluble	Methanol-soluble				Methanol-insoluble	^{14}C-bentazon
		A	B	C	D		
(days)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	2.5 a	7.1 a	5.4 a	26.1 a	18.6 a	7.6 a	33.9 b
5	5.5 b	9.7 a	7.6 a	34.8 a	13.2 a	15.4 b	13.4 a

[a]Means within columns with the same letter are not significantly different at the 5% level. Percent values were transformed to the arcsine for statistical analysis.

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

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Chapter 3

THE BASIS FOR BENTAZON SELECTIVITY IN NAVY BEAN, COCKLEBUR AND BLACK NIGHTSHADE

Abstract

The trifoliate leaf of tolerant navy bean (Phaseolus vulgaris L.) retained less bentazon (3-isopropyl-1H 2,1,3 benzothiadiazin-(4)3H-one 2,2 dioxide) than the unifoliate leaf, or the leaves from susceptible cocklebur (Xanthium pennsylvanicum Wallr.) and moderately susceptible black nightshade (Solanum nigrum L.) seedlings from foliar applications.

The ^{14}C from ^{14}C-bentazon applied to the foliage of black nightshade moved throughout the entire plant. In cocklebur, the ^{14}C moved throughout the treated leaf, whereas, in the trifoliate leaf of navy bean, very little acropetal movement occurred from the site of application. However, in the unifoliate leaf of navy bean, acropetal and basipetal movement of ^{14}C from the leaf was observed.

The species differed in the ^{14}C-metabolites formed from ^{14}C-bentazon 1 and 5 days after treatment. The rapid metabolism of bentazon in the trifoliate leaf of navy bean

appears related to tolerance.

Introduction

Bentazon is a potential postemergence herbicide for the control of broadleaf weeds in soybean (Glycine max (L.) Merr.) and navy bean not controlled by present preplant and preemergence herbicides[1] (1, 2, 5, 6, 7). Cocklebur is susceptible, and black nightshade moderately susceptible to bentazon. Both frequently escape currently used control measures in these crops.

Penner (4) related selectivity of bentazon between tolerant soybean and susceptible Canada thistle (Cirsium arvense L.) to less spray rentention on the leaf and a higher rate of bentazon metabolism in soybean.

The purpose of this study was to determine the basis for the selectivity of bentazon between navy bean at two stages of growth and two weed species, cocklebur and black nightshade, by comparing spray retention on the leaves, translocation and metabolism of bentazon.

Materials and Methods

"Sanilac" navy beans, cocklebur and black nightshade were grown from seed in a Conover sandy loam soil in the greenhouse at 25 ± 2°C with supplemental fluorescent lighting.

[1]Wyse, D.L., W.F. Meggitt and R.C. Bond. 1973. New herbicides for navy beans. Research Report N. Cent. Weed Contr. Conf. 28:76-79.

For the spray retention study, bentazon was applied at 2.24 kg/ha to cocklebur and black nightshade, having four to six leaves, and to navy bean having only unifoliate leaves or having both unifoliate and trifoliate leaves. After bentazon application, the leaves were immediately rinsed with distilled H_2O and leaf area determined. The rinse was evaporated to a 10 ml volume, acidified with 0.5 ml of concentrated HCl and extracted twice with 10 ml of ethyl acetate each. The ethyl acetate fractions were evaporated to dryness _in vacuo_, and the residue methylated in 1 ml of diethyl ether with diazomethane. The samples were then evaporated to dryness _in vacuo_, resuspended in 0.5 ml of ethyl acetate, and 10 μl analyzed quantitatively by comparison to standards with gas-liquid chromatography using a flame ionization detector and 4% SE-30 on gas chrom Q column. The temperature was 100°C, and the flow rate was 40 cc/min.

Procedure for the translocation and metabolism part of this study were the same as those previously reported (3).

A comparison was also made of the total amount of ^{14}C and that amount remaining as ^{14}C-bentazon in the leaf apices within each species with time. The total ^{14}C was computed by summing the total amounts contained by the ^{14}C-metabolites of the three solvent fractions, and that amount in the methanol-insoluble residue fraction. The ^{14}C remaining as bentazon was the sum present in the three solvent fractions.

Data presented in tables are the means of two experiments with three replications per experiment and three plants per replication. Plants shown in figures are representative of two or more experiments.

Results and Discussion

The trifoliate leaf of navy bean retained significantly less bentazon on the surface than was retained on the unifoliate leaf or the leaves of cocklebur and black nightshade seedlings (Table 1). These results indicate that differences in spray retention may contribute to bentazon selectivity between species.

More ^{14}C was translocated both acropetally and basipetally in unifoliate navy bean than in trifoliate navy bean (3). However, the ^{14}C movement in black nightshade seedlings was much greater than either and translocated throughout the entire plant (Figure 1). The movement increased with time. In cocklebur, the ^{14}C diffused acropetally throughout the entire leaf after 1 day (Figure 2). After 5 days the ^{14}C accumulated in the leaf apex.

As black nightshade is only moderately susceptible to bentazon, the extent of translocation does not appear related to tolerance. It may well be that the ^{14}C translocated is not ^{14}C-bentazon, but rather, readily translocated ^{14}C-metabolites not found in either navy bean or cocklebur.

The manner of bentazon metabolism in the unifoliate and trifoliate leaves of navy bean was similar (Table 2). However, in the unifoliate leaves of navy bean, a significantly greater percentage of ^{14}C remained as unmetabolized bentazon after 1 day (Table 3). Bentazon metabolism in black nightshade differed markedly from that in navy bean (Table 2). There were a greater number of metabolites in the ethyl acetate-soluble fraction and the R_f values of metabolites in both the ethyl acetate-soluble and methanol-soluble fractions differed from those in navy bean. However, the percentage of ^{14}C remaining as unmetabolized bentazon was significantly greater than in the trifoliate leaf of navy bean after 1 day (Table 3). Additional bentazon metabolism occurred in black nightshade between 1 and 5 days after treatment.

In cocklebur, more metabolites were also found 1 day after treatment than in the trifoliate leaf of navy bean (Table 2). As in black nightshade, numerous metabolites from cocklebur differed from those found in navy bean because of differences in R_f values and the greater quantity found in the ethyl acetate-soluble fraction. Again, the percent of ^{14}C remaining as unmetabolized bentazon was significantly greater in cocklebur than in trifoliate navy bean after 1 day (Table 3). Although the number of metabolites found in cocklebur increased after 5 days, the percentage of unmetabolized bentazon was much higher than in the trifoliate leaf of navy bean after 5 days (Table 2), or in any of the other plant material studied (Table 4).

The total amount of ^{14}C that accumulated in the leaf apex was significantly greater in cocklebur than in the other three types of plant material examined both 1 and 5 days after treatment (Table 4). This accumulation coupled with the slower rate of detoxication may also contribute to the susceptibility of cocklebur.

There was very little difference in the amount of unmetabolized bentazon remaining between the leaf apex of the unifoliate and the trifoliate leaves of navy bean after 1 day (Table 4). Since the ^{14}C was translocated more readily in the unifoliate leaves than in the trifoliate leaves of navy bean, apparently the bentazon distribution was greater, and this may relate to the previously observed greater susceptibility of navy bean treated when they had only the unifoliate leaves . The greater bentazon retention on the unifoliate leaves following postemergence applications may also contribute to the susceptibility.

Comparing the total ^{14}C and that amount remaining as unmetabolized bentazon in the leaf apex with time in each species, only cocklebur showed a significant increase in total ^{14}C and no significant change in ^{14}C remaining as bentazon after 5 days (Table 5).

Trifoliate navy bean was found to be tolerant to foliar applications of bentazon apparently because of less leaf retention and translocation, and an initially faster rate of metabolism when compared with unifoliate navy bean, cocklebur and black nightshade.

Black nightshade exhibited less susceptibility to foliar applications of bentazon than cocklebur. This could be partially attributed to the increased rate and extent of metabolism after 5 days. However, since significantly more ^{14}C

remained as unmetabolized bentazon in the leaf apex after 5 days compared with that amount remaining in the trifoliate leaflet apex of navy bean, there probably were other factors contributing to selectivity.

Extensive diffusion of bentazon throughout the leaf, slow metabolism, continued total ^{14}C accumulation in the leaf apex to 5 days after treatment and greater spray retention by the leaves all may have contributed to the observed susceptibility of cocklebur to foliar applications of bentazon.

Table 1. Leaf retention of bentazon after foliar application.[a]

Species	Amount retained (n moles/100 cm^2)
Navy bean unifoliate	1758 b
Navy bean trifoliate	802 a
Cocklebur	2167 b
Black nightshade	2410 b

[a]Means within the column having identical letters are not significantly different at the 5% level.

Figure 1. Translocation of ^{14}C-bentazon in black nightshade. The treated plants (A) and the corresponding radioautograph (B) show black nightshade with four to six leaves, harvested 1 day after foliar application. The treated plants (C) and corresponding radioautograph (D) show black nightshade at the same stage of growth harvested 5 days after foliar application.

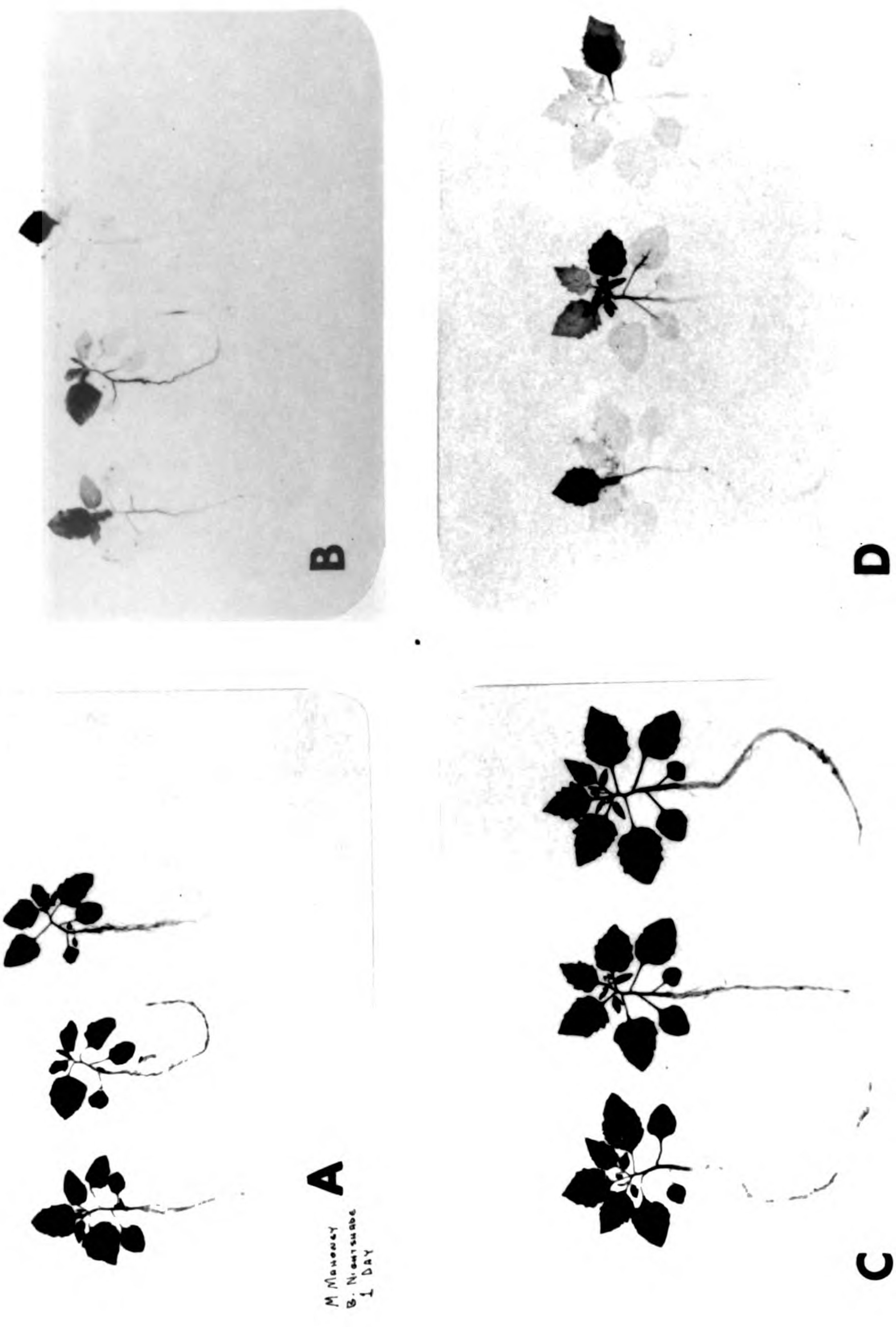
B
D
A
M Mahoney
B. Nightshade
1 Day
C

Figure 2. Translocation of ^{14}C-bentazon in cocklebur. The treated plants (A) and the corresponding radioautograph (B) show cocklebur with four to six leaves harvested 1 day after foliar application. The treated plants (C) and corresponding radioautograph (D) show cocklebur at the same stage of growth harvested 5 days after foliar application.

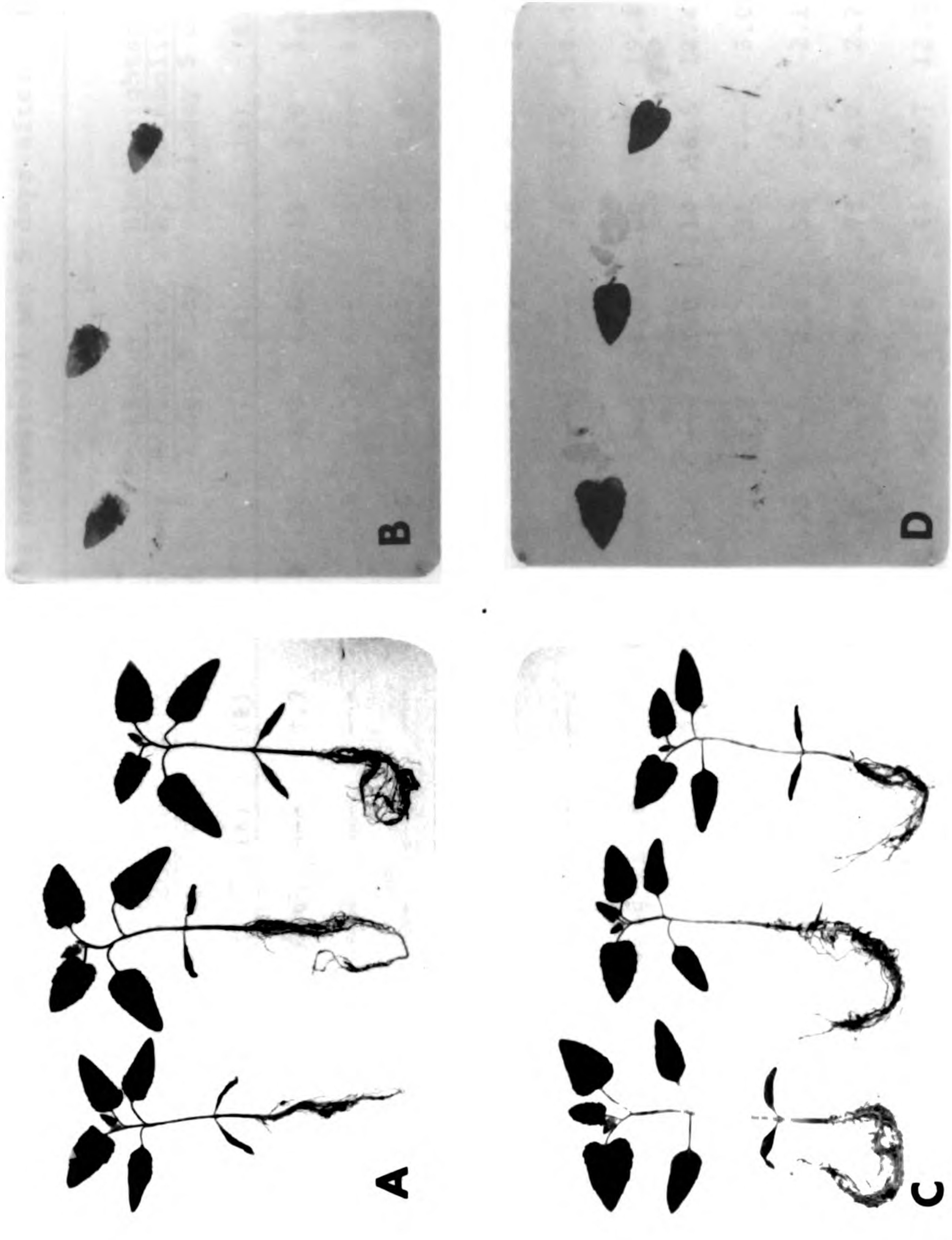
B
D
A
C

Table 2. Metabolites of ^{14}C-bentazon and corresponding R_f values obtained from leaf and leaflet apices of various plant species harvested 1 and 5 days after treatment.

Fraction	Navy bean					Cocklebur			Black nightshade		
	Unifoliate			Trifoliate							
	R_f	Metabolites		Metabolites		R_f	Metabolites		R_f	Metabolites	
		1 day	5 day	1 day	5 day		1 day	5 day		1 day	5 day
		(%)	(%)	(%)	(%)		(%)	(%)		(%)	(%)
Ethyl acetate-soluble	.18	0.6	---	0.7	1.4	.38	2.8	4.6	.15	2.0	1.1
	---	---	---	---	---	.49	1.3	---	.25	---	1.0
	---	---	---	---	---	.66	---	9.0	.50	2.8	7.6
	---	---	---	---	---	.72	14.4	0.5	.55	---	5.7
	---	---	---	---	---	---	---	---	.66	---	2.7
	---	---	---	---	---	---	---	---	.76	21.9	14.4
Water-methanol-soluble	.00	11.8	17.8	15.0	14.1	.00	8.2	6.0	.00	16.6	19.4
	.12	10.6	5.1	20.5	11.7	.14	---	3.0	.14	16.5	12.4
	.19	18.6	21.3	21.0	13.8	.42	4.2	17.2	.31	---	5.0
	.26	9.3	9.2	8.3	10.3	.73	---	2.8	.52	---	2.1
	---	---	---	---	---	.76	3.6	5.1	.78	4.2	2.7
Bentazon	.68	28.8	8.6	12.0	9.4	.65	61.4	44.0	.64	30.7	12.0

Table 3. Metabolism of ^{14}C-bentazon to the leaf apices of unifoliate and trifoliate leaves of navy bean, leaves of cocklebur and black nightshade 1 day after treatment.[abc]

Species	Origin	Metabolites	Methanol-insoluble-residue	Unmetabolized bentazon
	(%)	(%)	(%)	(%)
Navy bean unifoliate	11.1 b	39.1 b	21.0 b	28.8 b
Navy bean trifoliate	15.0 c	50.4 b	22.6 b	12.0 a
Cocklebur	8.2 a	26.0 a	4.4 a	61.4 c
Black nightshade	16.6 c	47.6 b	5.1 a	30.7 b

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

[b]Values were converted to the arcsine for statistical analysis.

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

Table 4. Comparison of ^{14}C-bentazon uptake and remaining unmetabolized bentazon between leaf apices of various plant species harvested 1 and 5 days after treatment.[a]

Species	1 Day			5 Day		
	Total	Unmetabolized bentazon		Total	Unmetabolized bentazon	
	(p moles/100 mg)	(%)	(p moles/100 mg)	(p moles/100 mg)	(%)	(p moles/100 mg)
Navy bean unifoliate	1,884 a	28.8	543 a	1,489 a	8.6	128 a
Navy bean trifoliate	4,676 ab	12.0	591 a	1,495 a	9.4	141 a
Cocklebur	12,343 c	61.4	7,577 b	24,537 c	44.0	10,796 c
Black night-shade	7,940 bc	30.7	2,438 a	11,898 b	12.0	1,428 b

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

Table 5. Comparison of total ^{14}C-bentazon accumulation and remaining unmetabolized bentazon from leaf apices of various plant species 1 and 5 days after treatment.[a]

Species	1 Day			5 Day		
	Total	Unmetabolized bentazon		Total	Unmetabolized bentazon	
	(p moles/100 mg)	(%)	(p moles/100 mg)	(p moles/100 mg)	(%)	(p moles/100 mg
Navy bean unifoliate	1,884	22.8	543**	1,489	6.6	128**
Navy bean trifoliate	4,676	12.0	591**	1,495	9.4	141**
Cocklebur	12,343*	61.4	7,577	24,537*	44.0	10,796
Black night-shade	7,940	30.7	2,438**	11,898	12.0	1,428**

[a]Means within rows with the same number of * across columns are significantly different at the 5% level.

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Chapter 4

SUMMARY AND CONCLUSION

Greenhouse and laboratory studies were conducted to examine spray rentention, translocation and metabolism of bentazon in seedlings of navy bean at two stages of growth, cocklebur and black niqhtshade. Studies were also conducted to examine bentazon translocation and metabolism in soybean.

The trifoliate leaves of navy bean retained less bentazon, metabolized ^{14}C-bentazon faster and showed less movement of ^{14}C from the site of application than the unifoliate leaves of navy bean, and the fourth leaf from the seedlings of cocklebur and black nightshade after 1 day. All of these factors appeared to play a role in the tolerance of navy bean to foliar applications of bentazon. Navy beans, with prior treatment of EPTC, chloramben and trifluralin, did not decrease the rate of bentazon metabolism. Only the trifoliate leaves of navy beans receiving prior treatment of EPTC showed greater translocations of ^{14}C. Navy bean tolerance to bentazon was not affected.

Greater translocation of ^{14}C and an increase in the amount of bentazon retained on the surface of the unifoliate leaves of navy bean appeared related to injury by foliar applications of bentazon at that stage of growth.

Continuing bentazon metabolism 1 to 5 days after treatment offers a partial explanation why black nightshade was only moderately susceptible. Other factors may also have contributed to the observed tolerance of this species to bentazon since there was a significantly greater amount of ^{14}C-bentazon in the leaf apex of black nightshade than in trifoliate navy bean.

Increased spray retention, more extensive acropetal movement, a slower rate of metabolism and a greater amount of ^{14}C remaining in the leaf apex 1 and 5 days after foliar applications of bentazon appeared related to cocklebur susceptibility to bentazon.

Translocation was more extensive and the rate of metabolism was slower in soybean than in navy bean after 1 day. Other factors not studied may also have contributed to the tolerance of soybean to foliar applications of bentazon.

Soybean harvested at maturity contained no ^{14}C-bentazon. Four apparent ^{14}C-conjugates of bentazon were isolated with molecular weights ranging from approximately 269 to 698.

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