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HERBICIDAL ACTIVITY AND EFFICACY ON
BLACK NIGHTSHADE (*SOLANUM* *spp.*)
IN NAVY BEANS (*PHASEOLUS* *VULGARIS* L.)

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John Wesley Vandeventer

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By

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ABSTRACT

HERBICIDAL ACTIVITY AND EFFICACY ON

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IN NAVY BEANS (*PHASEOLUS VULGARIS* L.)

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Field studies at three locations were utilized to determine the effects of selected preplant incorporated herbicide treatments on black nightshade (*Solanum sp.*), navy bean (*Phaseolus vulgaris* L.), and herbicide persistence measured by soil residue analysis and activity on wheat (*Triticum aesitivum* L.). As temperature at application increased, the herbicides were more efficacious on black nightshade, similarly as organic matter level decreased the same was true. No major differences due to herbicide usage were seen on navy bean yield or in wheat stand and yield the following season. No differences were seen in persistence between ethalfluralin (*N*-ethyl-*N*-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) and trifluralin (a,a,a-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) in the soil for a period of 470 days.

Black nightshade is an extremely variable species and when plants grown from two seed sources were compared on a morphological and physiological basis vast response differences were observed. Plants grown from seed obtained in Michigan (MI) had a yellow anther column in the

flower and toothed proximal portion on the mature leaf while those plants grown from a California (CA) seed source had a dark brown anther column in the flower and an entire leaf margin. The plants from the CA seed source were more vigorous over a 15 to 30 C temperature range. Alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide) was the most efficacious on black nightshade and better black nightshade control resulted from ethalfluralin than trifluralin. These differences in black nightshade control among substituted dinitroaniline herbicides and between seed sources were not explained by differential ^{14}C absorption or distribution. Herbicide metabolism studies involving ^{14}C indicated that differences may be due to the more rapid metabolism of ^{14}C-ethalfluralin than ^{14}C-trifluralin. Metabolism was more rapid in the plants grown from the CA seed source than MI seed source. These observations appear related to the differences in control in the field and greenhouse and also account for the herbicidal activity levels observed in plants from the MI and CA seed sources.

To the beautiful woman that stands with me always, Nancy Ann.

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INTRODUCTION

Black nightshade (*Solanum sp.*) has long been a problem for navy bean farmers. Much has been written about the extreme variability within the genus. Different descriptions, both written and pictorial, can be found of *Solanum nigrum* L. Proper weed control measures are dependent upon accurate identification of the pest species.

Problems caused by black nightshade are not only competition but also the discoloration of navy beans at harvest due to the staining juice present in the black nightshade fruit. The seriousness of this problem may warrant the use of additional herbicides. Problems arise when weed control recommendations are based on other than native weed populations. In some cases even the native populations vary genetically.

Black nightshade control options include the use of preplant incorporated herbicides alone or in combination with other preplant incorporated or preemergence herbicides. The best practice would be to maximize black nightshade control and crop yield while minimizing herbicide persistence in the soil and carry over problems for rotational crops. The substituted dinitroaniline herbicides and alachlor have been the primary choices for use by the navy bean farmer.

These studies were undertaken to compare and evaluate (1) selected herbicides for black nightshade efficacy, activity on navy beans and wheat, soil persistence between ethalfluralin and trifluralin, (2) the morphological and physiological variability of black nightshade plants grown from two seed sources as related to their identification, and (3) the basis for difference in control with ethalfluralin and trifluralin applied to plants from two seed sources.

CHAPTER 1

EFFICACY ON BLACK NIGHTSHADE (SOLANUM AMERICANUM MILL.) AND ACTIVITY OF PREPLANT INCORPORATED HERBICIDES FOR WEED CONTROL IN NAVY BEANS (PHASEOLUS VULGARIS L.)

ABSTRACT

Dinitroaniline herbicides and alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide) efficacy on black nightshade (Solanum americanum Mill.) native to Michigan and activity on navy beans (Phaseolus vulgaris L.) and wheat (Triticum aesitivum L.) were examined. Field results indicated that regardless of soil type the most consistent control of black nightshade was obtained from preplant incorporated alachlor. Addition of chloramben (3-amino-2,5-dichlorobenzoic acid) or dinoseb (2-sec-butyl-4-,6-dinitrophenol) as a preplant incorporated tank mix or preemergence overlay treatment did not increase navy bean yield in all cases. As the soil organic matter increased, less black nightshade control was obtained from the addition of chloramben regardless of preplant incorporated treatment. At one location no difference in navy bean yield was seen from the addition of chloramben applied preemergence to almost any preplant incorporated dinitroaniline herbicide except for dinitramine (N^4,N^4-diethyl-a,a,a-trifluoro-3,5-dinitrotoluene-2,4-diamine) where yields increased more than 40%. Decreased navy bean yields at this same location resulted from the chloramben addition to alachlor applied

preplant incorporated. At a lower organic level, location III, highest navy bean yields resulted from fluchloralin (N-(2-chloroethyl)-2,6-dinitro-N-propyl-4-(trifluoromethyl)benzenamine) at 1.68 kg/ha and ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) at both rates tested (1.12 and 1.68 kg/ha).

No differences were seen in wheat stand or yield resulting from dinitroaniline carry over at location I. No differences in concentration of herbicide residue in the soil were found between ethalfluralin and trifluralin.

INTRODUCTION

Black nightshade (Solanum americanum Mill.) frequently listed as S. nigrum L. has long been a serious problem to navy bean (Phaseolus vulgaris L.) farmers. Until recently options for effective nightshade weed control in Michigan with preplant incorporate tank mix treatments consisted of EPTC (S-ethyl dipropylthiocarbamate) plus trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine), dinitramine (N^4,N^4-diethyl-a,a,a-trifluoro-3,5-dinitrotoluene-2,4-diamine), or profluralin (N-cyclopropylmethyl)-a,a,a-trifluoro-2,6-dinitro-N-propyl-p-toluidine) plus chloramben (3-amino-2,5,-dichlorobenzoic acid) (7). Preplant incorporated plus preemergence herbicide treatments consisted of EPTC or a substituted dinitroaniline herbicide followed by chloramben or dinoseb (2-sec-butyl-4-,6-dinitrophenol). The weed control with preplant incorporated tank mix treatments may be more than from the same preplant incorporated plus preemergence treatments under dry conditions. If rainfall, is received within a short time (4 to 7 days) following application, greater weed control is obtained from the preplant incorporated plus preemergence treatments.

Volatility is an important factor affecting substituted dinitroaniline herbicide biological activity. Conditions conducive to volatilization include high soil moisture and high temperature (1). As the soil moisture increased, trifluralin vaporization increased (15). The vapor from most of the substituted dinitroanilines is herbicidally active and diffusion is the greatest under dry soil conditions (1). Soil organic matter has also been mentioned as an important factor in reducing substituted dinitroaniline herbicide activity (3,4,8). Variation in soil persistence from substituted dinitroaniline herbicides has been reported (11,13,16). The persistence of substituted dinitroaniline herbicides depends on climatic and edaphic factors that affect their rate of disappearance (12). Results from Saskatchewan indicate that trifluralin applied at the 1 to 2 kg/ha rate persisted after one growing season and declined during the first months of the second season to very low levels after 2 months.

Ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) is considered to be less persistent in the soil and offers greater black nightshade control than trifluralin (6,14). The combination of alachlor (2-chloro-2',6'-diethyl-N-methoxymethyl) acetanilide) plus chloramben (preplant incorporated has similar characteristics.

The objectives of this study were to evaluate: 1) substituted dinitroaniline herbicide and alachlor efficacy on black nightshade; 2) effect on navy bean yield from preplant incorporated herbicide treatments alone and in combination with chloramben and dinoseb applied in a preplant incorporated tank mix or as a preemergence overlay; 3) effect of substituted dinitroaniline herbicide persistence on wheat stand and yield; and 4) ethalfluralin and trifluralin degradation over time.

MATERIALS AND METHODS

A two year efficacy and persistence study was initiated in June, 1976 in Sanilac Co., MI (location I). Herbicides given in Table 1 were applied to a sandy clay loam. Treatments were applied in a randomized complete block design with three replications. Plot size was 3 m by 15.25 m.

All treatments were applied with a tractor mounted sprayer at the rate of 215 l/ha and 2.1 kg/cm^2. All substituted dinitroaniline herbicides were incorporated to a uniform 7.5 cm depth with a spring-tooth harrow two times at right angles.

Preemergence overlay was applied after planting with "Seafarer" navy beans on June 21. Identical treatments were applied on June 12 at Huron Co. (location II). These plots (location II) were only used for black nightshade efficacy. In 1977, repeat studies were put out in both locations I and II on June 12 and 17, respectively. In June 1977, studies were initiated to evaluate the effectiveness of these same substituted dinitroaniline herbicides and alachlor at various rates both alone and in combination with chloramben (locations I and II) and dinoseb (location III, Gratiot Co., MI) in an applied preplant incorporated tank mix and as preemergence overlays.

At location I soil samples were taken from each ethalfluralin and trifluralin plot on seven dates, June 21, July 20, August 22, September

30, 1976 and the following April 28, August 5, and October 7, 1977. All samples were taken with a 19 mm sampling tube to a depth of 15 cm and consisted of 20 subsamples. After each date, samples were shipped to Lilly Research Laboratories, Greenfield, IN for analysis using Eli Lilly and Company Agricultural and Analytical Chemistry Procedure No. 5801616 for trifluralin and Procedure No. 5801633 for ethalfluralin (2,5).

After navy bean harvest at location I, "Tecumseh" wheat (Triticum aesitivum L.) was planted on October 3 as a rotational bioassay crop. The following spring wheat density measurements (plants/m^2) were taken and on July 12, 1977 the wheat was harvested with a small plot combine for yield data.

A split-plot design with four replications was utilized at location III to study the substituted dinitroaniline herbicides and alachlor (Table 1) alone and in preplant incorporated tank mix and preemergence combinations with chlorambem and dinoseb. Treatments were applied on June 14 and 15 and "seafarer" navy beans were planted on June 15, 1977. This location was originally thought to have a uniformly dense black nightshade population. Weed control ratings were made at 40 days, however, the nightshade population was so variable, it was not included in the ratings. Navy bean yields were taken at harvest.

Temperature data for the 4 days prior to treatments as an indication of soil temperature is included in Table 2 for locations I and II. Rainfall data is shown in Table 3 for locations I and II for June, 1976 and locations I, II and III for June 1977 (9.10).

All yield and stand data are expressed as percent of untreated control. Soil residues are reported in μg/g. Thirty or 40-day weed control ratings were made on a 0 = no control, 10 = complete control scale and are expressed as percent control. Soil mechanical analysis for locations I and II and percent organic matter for locations I, II and III may be found in Table 4.

RESULTS AND DISCUSSION

No differences in black nightshade control between locations I and II was evident in 1976. The most efficacious treatments in 1976 were trifluralin at 1.4 kg/ha, dinitramine at 0.56 kg/ha, ethalfluralin at 1.68 kg/ha, and pendimethalin at 1.4 kg/ha (Table 5). The temperatures for the preceeding 4 days prior to treatment averaged 7 C higher at location II than I, the organic matter (O.M.) was also different, location I = 9.46% and location II = 6.71%. The effects of temperature and organic matter may have cancelled each other; location I - lower temperatures and a higher O.M., whereas location II - higher temperatures and a lower O.M. (Table 2).

In 1977 the temperatures prior to treatment were 3 C higher for location II. The rainfall received within 7 days of treatment was 0.05 cm for location I and 1.1 cm for location II. The increased rainfall could have provided the stimulus for germination along with the warm temperatures to provide for active growth of weed seedlings in the treated soil zone giving a significant location by treatment interaction. Better weed control (Table 5) was seen from four of eleven treatments. The combination treatments in which chloramben preemergence increased black nightshade control were trifluralin at both rates (0.86 and 1.4 kg/ha), profluralin at 1.12 kg/ha, and fluchloralin at 1.68 kg/ha (Table 5). This increased weed control was probably due to the chloramben because these three herbicides normally do not control black nightshade. Chloramben application at location I did not increase the level of black nightshade whereas at location II better black nightshade control was seen from herbicides in combination with chloramben. This difference in activity may be due to the level of organic matter in these two soils. As the percent organic matter increased the amount bound or unavailable for plant uptake was decreased.

The combination study at location III showed no navy bean yield differences between any addition to the preplant incorporated treatments, chloramben as preplant incorporated tank mix or preemergence, or dinoseb at preplant incorporated tank mix or preemergence. The treatments resulting in the highest navy bean yields were ethalfluralin (1.68 and 1.12 kg/ha) and fluchloralin (1.68 kg/ha) (Table 6).

In 1976 there were no significant navy bean yield differences between treatments. During 1977 significant differences in yields due to chloramben addition were seen in three cases (Table 6). Navy bean yield after treatment with dinitramine compared to dinitramine plus chloramben was increased by 46%. Adding chloramben to alachlor decreased yields in both the low and high alachlor rates by at least 45%. No significant yield differences were seen from the remaining substituted dinitroaniline herbicide plus chloramben treatments.

Soil persistence of the substituted dinitroaniline herbicide treatments applied in 1976 at location I was measured with wheat, a likely rotational crop. There was no significant stand or yield effect seen from any substituted dinitroaniline herbicide treatment on wheat stand or wheat yield both reported as percent untreated control (Table 7).

Soil persistence over 1 1/2 years previous was determined at location 1 for ethalfluralin and trifluralin. The first sampling date was immediately following application. The second through fourth dates followed at approximately 30 day intervals. There were no significant differences between persistence at the first two dates or the second through fourth dates (Table 8). Therefore, presuming extractable herbicide can be related to potential phytotoxicity, no decreased activity was seen in the 30 day through the 90 day samplings. A significant decrease in herbicide persistence was seen over the first 60 day period. Samples collected the following spring (310-470 days after application) were not significantly different. However, lower residue levels were observed one year after application.

During the first 60 days the most dramatic decrease in soil persistence is usually seen. Initial losses were observed to be greater than subsequent losses. The most dramatic losses occurred during the overwinter period and in the early spring when excess soil moisture favored volatilization.

Ethalfluralin residues at both tested rates (1.12 and 1.68 kg/ha) were the same as that for trifluralin at the 0.84 kg/ha rate over the 470 day period. Trifluralin at the 1.4 kg/ha degraded over the time period similarly to ethalfluralin at the 1.68 kg/ha rate. Ethalfluralin was not found to be less persistent with respect to extractable quantities over time than trifluralin regardless of rate used. The t 1/2 or point in time at which one half the amount originally sampled was found for all the four treatments was the fifth date or the following spring.

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Table 1. Herbicide treatments applied at locations I, II, and III during 1976 and 1977.

Year					
1976		1977			
Loc I and II[a]		Loc I and II[b]		Loc III[c]	
Herbicide	Rate (kg/ha)	Herbicide	(kg/ha)	Herbicide	Rate (kg/ha)
Trifluralin	0.84	Trifluralin	0.84	Trifluralin	0.84
	1.40		1.40		1.40
Profluralin	1.12	Profluralin	1.12	Profluralin	1.12
Fluchloralin	1.68	Fluchloralin	1.68	Fluchloralin	1.68
Dinitramine	0.56	Dinitramine	0.56	Dinitramine	0.56
Butralin	1.68	Butralin	1.68	Butralin	1.68
Ethafluralin	1.12	Ethalfluralin	1.12	Ethalfluralin	1.12
	1.68		1.68		1.68
Pendimethalin	1.40	Pendimethalin	1.40	Pendimethalin	1.40
		Alachlor	2.24	Alachlor	2.24
			2.80		2.80

[a]Treatments applied preplant incorporated with a preemergence chloramben 2.24 kg/ha overlay.

[b]All treatments were applied both alone preplant incorporated and preplant incorporated with a preemergence chloramben 2.24 kg/ha overlay.

[c]All treatments applied alone preplant incorporated and in combination with chloramben at 2.24 (preplant incorporated tank mix and preemergence) and with dinoseb 5.04 kg/ha (preplant incorporated tank mixed and preemergence).

Table 2. Mean maximum air temperatures for four days prior to herbicide treatment at locations I and II and corresponding overall black nightshade percent control and percent organic matter (O.M.).

	Location					
	I			II		
Year	Temperature (C)	O.M. (%)	Mean blns ctrol (%)	Temperature (C)	O.M. (%)	Mean blns ctrol (%)
1976	24	9.46	59	31	6.71	75
1977	17	9.46	52	20	6.71	64

Table 3. Rainfall at locations I and II for June, 1976 and for locations I, II, and III for June, 1977.

Year						
1976			1977			
	Location			Location		
Day	I (cm)	II	Day	I	II (cm)	III
1	0.23		1	0.97	0.74	0.58
2			2	0.71	1.14	0.79
3			3	T	0.05	
4			4			
5			5	0.61	0.89	0.05
6			6	0.79	1.04	0.57
7			7	0.13	1.02	0.66
8			8	0.41		
9		T	9	0.46		
10			10			
11		T	11	0.20		
12			12	0.33	0.18	0.03
13			13	0.08		T
14			14			
15			15			
16	0.03	0.79	16			
17			17		0.10	0.03
18			18	0.58	0.84	1.02
19	2.16	0.97	19		0.05	
20	1.04		20	0.03		
21			21		0.08	
22		T	22			
23			23			
24		0.05	24			
25	0.79	0.20	25			
26		T	26		0.74	
27			27			
28			28		0.71	
29	3.12		29	0.10		1.14
30	1.73		30			

Table 4. Soil mechanical analysis for locations I and II and percent organic matter for locations I, II and III.[a]

Location	Organic Matter (%)	Mechanical Analysis Sand (%)	Silt (%)	Clay (%)	Texture
I	9.46	52.4	20.72	26.88	sandy clay loam
II	6.71	76.40	6.72	16.88	sandy loam
III	5.0	--	--	--	sandy loam

[a]All soil analyses conducted by the Michigan State University Soil Test Laboratory, 108 Soil Science Building, East Lansing, MI 48824.

Table 5. Effect of herbicide treatment on percent black nightshade control at locations I and II during 1976 and 1977.

Year						
1976[a]			1977			
					Location Black nightshade (%C)	
Herbicide[b]	Rate (kg/ha)	Black nightshade (%C)	Herbicide	Rate (kg/ha)	I	II
Trifluralin	0.84	57 a[c]	Trifluralin	0.84	33 ab	78 d-g
	1.40	66 abc		1.40	28 a	87 d-g
Profluralin	1.12	62 ab	Profluralin	1.12	28 a	81 d-g
Fluchloralin	1.68	62 ab	Fluchloralin	1.68	40 abc	80 d-g
Dinitramine	0.56	80 c	Dinitramine	0.56	54 a-d	79 d-g
Butralin	1.68	62 ab	Butralin	1.68	63 b-g	58 a-e
Ethalfluralin	1.12	63 ab	Ethalfluralin	1.12	60 a-f	72 c-g
	1.68	75 bc		1.68	77 d-g	68 c-g
Pendimethalin	1.40	78 bc	Pendimethalin	1.40	70 c-g	95 fg
			Alachlor	2.24	96 fg	93 efg
				2.80	82 d-g	98 g

Combinations

	Black nightshade (%)	
Location	w/o chloramben	w/ chloramben
I	52 a	63 ab
II	66 b	96 c

[a]Black nightshade control values are means of both locations I and II.
[b]All herbicide treatments are in combination with chloramben (2.24 kg/ha) applied preemergence.
[c]Means within years and section followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Effect of herbicide application on dry bean yield at location I and location III in 1977.

Herbicide	Rate (kg/ha)	I yield[b] w/o chloramben (%C)[d]	I yield[b] w/ chloramben (%C)[d]	III[a] yield[c] (%C)[d]
Trifluralin	0.84	174 b-g	186 c-h	152 bc
	1.40	174 b-g	163 a-d	151 bc
Profluralin	1.12	185 c-h	161 abc	137 ab
Fluchloralin	1.68	174 b-g	180 b-g	157 bcd
Dinitramine	0.56	171 b-f	218 h	140 ab
Butralin	1.12	145 ab	160 abc	142 ab
Ethalfluralin	1.12	170 b-c	202 e-h	165 cd
	1.68	176 b-g	208 gh	172 d
Pendimethalin	1.40	188 c-h	162 abc	151 bc
Alachlor	2.24	198 d-h	134 a	147 bc
	2.80	206 fgh	161 abc	147 bc

[a]Yield means are the average for treatments alone and in combination with chloramben 2.24 kg/ha, preplant incorporated tank mix and preemergence, and dinoseb 5.04 kg/ha, preplant incorporated tank mix and preemergence.
[b]No treatment dry bean yield at location I in 1977 was 788 kg/ha (7.03 cwt/A).
[c]No treatment dry bean yield at location III in 1977 was 788 kg/ha (7.03 cwt/A).
[d]Means within locations followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 7. The effect of substituted dinitroaniline carry over on wheat stand density and yield (both expressed as % untreated control).

Herbicide	rate (kg/ha)	Wheat stand density	Wheat yield
Trifluralin	0.84	109	86
	1.40	94	88
Profluralin	1.12	102	98
Fluchloralin	1.68	70	89
Dinitramine	0.56	96	94
Butralin	1.68	99	92
Ethafluralin	1.12	96	104
	1.68	89	71
Pendimethalin	1.40	98	99

Table 8. Herbicide residue in soil following application of substituted dinitroaniline herbicides during 470 days at location I.

Sampling date	Residue level[ac] (μg/g)	Herbicide	Application (kg/ha)	Residue level[bc] (μg/g)
6/21/76	0.44 c	trifluralin	0.86	0.22 a
7/20/76	0.33 bc		1.40	0.31 b
8/22/76	0.29 b	ethafluralin	1.12	0.19 a
9/30/76	0.31 b		1.68	0.26 ab
4/28/77	0.14 a			
8/5/77	0.13 a			
10/7/77	0.07 a			

[a]Residue levels are the mean of four treatments and both herbicides.
[b]Residue levels are the means of seven sampling dates.
[c]Means within columns followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

CHAPTER 2

MORPHOLOGICAL AND PHYSIOLOGICAL VARIABILITY IN BLACK NIGHTSHADE (*SOLANUM* *spp.*)

ABSTRACT

Plants from two black nightshade seed sources were compared for differences in morphology at seedling, mature plant, and flowering and physiological response to pregermination seed treatment, growth responses to temperature, and herbicide response.

Differences in morphology of these two *Solanum* *spp*. were evident from seedling through maturity. The plants grown from the MI seed source had a deep purple abaxial leaf surface at 3 to 4 leaf stage and a yellow anther column and toothed proximal half in the mature leaf whereas the plants grown from the CA seed source had no unusual abaxial coloration or toothed proximal margin and had a dark brown anther column. From this point differing responses to treatment between seed source were evident. Throughout the temperature studies, plants from the CA seed source were more vigorous. Of the herbicides evaluated, very few controlled black nightshade. Differences in response to herbicides were seen between plants grown from the two seed sources following preplant incorporated, preemergent and postemergent herbicide application. The greatest differences in plant growth were with chloramben (3-amino-2,5-dichlorobenzoic acid) applied preplant incorporated at 1.68 kg/ha and ethalfluralin

(N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) applied preemergence at 1.12 kg/ha where plants from the MI seed source were almost always at least 5 times more susceptible than plants from the CA seed source. Plants from two black nightshade seed sources have been described as Solanum nigrum L. However, differences in morphology and physiology indicated genetic differences. The plants from MI have been identified as Solanum americanum Mill. and those from CA as Solanum scabrum Mill. This correct identification could resolve conflicting reports among researchers regarding chemical control measures.

INTRODUCTION

The Solanum spp. is recognized as having great variability causing taxonomic difficulties (13). The most widespread species group in the complex is Solanum nigrum L. S. nigrum L. is reported as a weed in 61 countries and in 37 crops. The common name associated with the S. nigrum L. in the United States is black nightshade (9). Black nightshade, often reported as S. nigrum L., has been reported to be a serious pest in row crops including sugar beets (Beta vulgaris L.) (14), soybeans (Glycine max L. Merr.) (2,10), lima beans (Phaseolus limensis L.) (3), corn (Zea mays L.) (9,18) and dry beans (Phaseolus vulgaris L.) (6,7,16).

Temperatures between 25 and 30 C (4) and more specifically 30 C are optimum for germination and for seedling growth (5). Germination was significantly affected by planting depth. Depths below 0.25 cm significantly reduced the percent germination regardless of soil type (5).

In an earlier study (17), black nightshade seed was obtained from a California (CA) based seed supplier in the spring of 1976 and seed was obtained from Michigan fields (MI). After growing plants under identical greenhouse conditions, morphology of plants from the MI seed sources resembled the S. nigrum L. seen in Weeds of the North Central States (15) and Nebraska Weeds (11), while the morphology of the CA seed source resembled S. nigrum L. from World's Worst Weeds (9) (Figure 1).

The objectives of this study were to compare morphology of these two black nightshade seed sources regarding their seed, seedling, and mature stages and to study their physiology with regard to germination and its inhibition, growth response to temperature, and responses to preplant incorporated, preemergence and postemergence herbicide treatments.

MATERIALS AND METHODS

Fruits from native (MI) black nightshade (Solanum sp.) were collected in Sanilac Co., MI from the soil surface after the fruits had fallen. Fruits were air dried at 22 C and seeds separated from the dried fleshy portion and stored under refrigeration (4 C) until 48 h prior to use. Pre-cleaned black nightshade (Solunum sp.) seed was obtained from a California (CA) based seed supplier (Valley Seed Co., Fresno) and stored under identical conditions until 48 h prior to use.

For the morphology study, seeds from both the MI source and CA source were compared at 30X magnification. Seeds from both sources were planted at 0.5 cm depth and seedlings allowed to grow in the greenhouse with natural light and supplemental fluorescent lighting 16 h/day at 25 $\pm$ 3 C. At seedling, mature plant, and flowering stages morphological comparisons were made with regard to size, coloration (stem and leaf adaxial and abaxial surfaces) leaf veination and shape, and floral characteristics.

For the physiology study, germination was examined with seeds from both MI and CA sources from plants grown under identical conditions, then subjected to: 1) no pre-treatment; 2) soaked in 1, 10, 25, 50, and 100 ml of distilled water for 24 h; and 3) washed in running tap water (11 C) for 1, 2.5, 5, 7.5, 12, 48, and 108 h. After the above mentioned, seeds were placed on wet filter paper in plastic petri dishes, sealed with parafilm and incubated in a lighted growth chamber (16 h/day) at 25 C for 14 days and germination percentage determined.

To determine whether plants from the two seed sources responded differently to temperature, growth chamber studies were run at 15, 20, 25 and 30 C and percent emergence and seedling growth were evaluated. Florescent/incandescent supplemental lighting (16 h/day) was used and surface irrigation was provided as necessary.

To determine whether the plants from the two seed sources responded similarly to herbicides, the herbicides listed in Table 1 were selected on their potential field use at use rates for preplant, preemergence and postemergence application to navy beans or use in the navy bean crop rotation (Table 2).

Preplant incorporated and preemergence herbicide treatments were applied to a greenhouse soil mix (3 soil:1 sand) with approximately 7.5% organic matter. All herbicides were applied with a link belt sprayer at 2.1 kg/cm^2 in 280 l/ha. All preplant incorporated treatments were incorporated in a mechanical rolling mixer. Fifteen seeds were planted per 946 ml waxed paper cup at 0.5 cm depth.

Postemergence treatments were applied to four plants/cup at the one true leaf stage and harvested 3 weeks later.

All plants were grown in the greenhouse with surface irrigation, supplemental lighting (16 h/day), and at 25 C. Treatments were randomized in a complete block design with four replications. All data reported are the means of two experiments.

RESULTS AND DISCUSSION

As seen in Figure 2, the seeds from MI are approximately 1.5 to 1.7 mm by 0.9 to 1.0 mm while the seeds from CA are much larger being approximately 2.5 to 2.8 mm by 1.5 mm. When viewed at a magnification of 30X seed from both sources had a rough appearing seed coat that was coarsely grained and pitted. The seed from the MI source had a yellow-golden tan coloration while the seed from the CA source had a charcoal-grey black coloration. Seed from both sources had the same exterior shape and form.

At the cotyledon stage of development no apparent differences in plant morphology were evident. When the seedlings had two to four leaves, differences in the coloration of the abaxial surface of the leaves were evident (Figure 3). Plants from the MI source had prominent veination and a deep purple color on the abaxial side while plants from the CA seed retained a green color on the abaxial side. The stems of the plants from the MI source also had similar purple coloration as the abaxial leaf surface. Seedlings from the CA source appeared more vigorous than those from the MI source.

At maturity, the leaves from plants from the two sources no longer exhibited the difference in abaxial coloration but did have differing leaf margins (Figure 4). Plants from the MI source had an ovate shape with a distinct serrated margin on the proximal half. Plants from the

CA source had an ovate leaf with uniformly entire margin.

Flowers from plants from the MI seed source and CA seed source were clustered in groups of two to five and three to seven, respectively, and had a calyz of five united sepals; white petals were on those from the CA source or white tinged with purple from the MI seed source. The anthers were united in plants from both sources. Anthers from the MI seed source formed a yellow column while those from the CA seed source formed a dark brown column (Figure 5).

The description for the plants grown from the MI seed source resembled that given for S. nigrum L. in both Weeds of the North Central States (15) and The World's Worst Weeds (9). The description for the plants from the CA seed source stated above with the exception of the dark brown united anthers also resembled S. nigrum L. in The World's Worst Weeds. The plants grown from the MI seed source, however, did resemble the description (1) for Solanum americanum Mill. The morphological differences indicated that we may possibly be examining other species entirely.

The effect of the pre-germination seed treatment also showed differences with regard to seed source (Table 3). No pre-treatment at all resulted in no significant difference between sources and a low germination percentage from both sources (MI 5%, CA 18%). Although there were no differences due to the soaking volumes over 24 h, the germination response to 24 h soaking was significant for the CA source. The percent germination of the MI seed was significantly less, when soaked for 24 h, indicating genetic differences between sources.

The germination study using a running tap water bath over time resulted in a significant seed source by time interaction. We expected an increase in the percent germination with time considering that a germination inhibitor may have caused low germination. However, this was not the case as the longer the seed from the two sources was in the 11 C bath, the lower the percent germination (Table 4). From 1 h to 108 h after soaking the germination percentage of seed from the MI source decreased from 76 to 52%, whereas the germination percentage of seed from the CA source decreased from 68 to 22% (Table 4). Germination may have been reduced with time by the low temperature and oxygen content of the tap water.

The response of plants from the two sources to temperature as measured by percent germination and plant height showed a significant temperature by source interaction for germination. Plant height varied with seed source (Table 5). Seeds from the MI source failed to germinate at 15 C compared to the seed from the CA source with 49% germination. Seed from the CA source germinated especially well over the range of 15 to 30 C. Plants from both sources showed increased plant height with increased temperature. No differences were observed between or among temperature or source with respect to dry weight/plant (Table 5).

Plants from the two sources also responded differently to the selected preplant incorporated herbicide treatments (Table 6). The most pronounced difference was observed with respect to plant number, plant height and dry weight/plant (all as percent of control) following treatment with chloramben at 1.68 and 2.24 kg/ha. Plants from the MI seed source were much more susceptible to chloramben. Ethalfluralin showed more activity on plants from both sources than trifluralin.

Preemergence application of chloramben alone or in combination with alachlor was equally effective on plants from the two sources (Table 7). Plants from the CA source appeared more tolerant to ethalfluralin and metolachlor than plants from the MI source. This is not significant for ethalfluralin as this herbicide is usually preplant incorporated. At both rates of bentazon tested, the postemergence application appeared less effective on the plants from the MI source with regard to plant height and dry weight/plant (Table 8).

Responses observed in plants grown from the MI seed source are generally consistent with those observed in Michigan State University field trials. Preplant incorporated herbicides applied to seeds planted from both sources were generally ineffective with the exception of alachlor and alachlor plus chloramben at all rates tested. In addition to the aforementioned herbicide treatments, ethalfluralin and dinitramine have generally resulted in black nightshade control in Michigan. A possible reason for this may be that consistent watering in the greenhouse compared to infrequent natural rainfall provided a soil atmosphere conducive to herbicide loss resulting in reduced black nightshade efficacy, except when ethalfluralin was applied as a preemergence treatment. Under field conditions, ethalfluralin applied as a preemergence herbicide has not resulted in good black nightshade control at these tested rates. Reasons for this may be reduced field efficacy caused by chemical volatility and photodecomposition caused by ultra-violet light.

Postemergence herbicide treatments applied to plants grown from both the MI and CA seed sources were generally ineffective. Similar results for native MI black nightshade have been reported in field studies.

Both the morphological and physiological studies showed marked differences between plants from the two seed sources. If both were *S. nigrum* as classified (15,9), then conflusion in their control by various herbicides may be explained. Confusion could be avoided if these plants were reclassified. After careful systematic examination Schilling (12) has suggested that neither plant from these two sources be classified as *S. nigrum* and that the plants from the MI source were *S. americanum* Mill. and plants from the CA source were *S. scabrum* Mill. Future use of this classification system should help avoid confusion.

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Table 1. Common and chemical names of herbicides used.

Common name	Chemical name
Alachlor	2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide
Bentazon	3-isopropyl-1H-2,1,3-benzothiadiazin-4-(3H)-one 2,2-dioxide
Chloramben	3-amino-2,5-dichlorobenzoic acid
Desmedipham	ethyl m-hydroxycarbanilate carbanilate (ester)
Dinitramine	*N*^{4},*N*^{4}-diethyl-a,a,a-trifluoro-3,5-dinitrotoluene-2,4-diamine
Ethalfluralin	*N*-ethyl-*N*-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine
Ethofumesate	2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulphonate
Metolachlor	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide
Phenmedipham	methyl m-hydroxycarbanilate m-methylcarbanilate
Trifluralin	a,a,a-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine

Table 2. Preplant incorporated, preemergence and postemergence herbicides selected for response comparison between two black nightshade seed sources.

Application Method					
Preplant incorporated		Preemergence		Postemergence	
Herbicide	Rate(s) (kg/ha)	Herbicide	Rate(s) (kg/ha)	Herbicide	Rate(s) (kg/ha)
ethalfluralin	1.12, 2.24	alachlor	1.68 + 2.24	bentazon	0.56, 1.12
trifluralin	0.84	metolachlor	2.24, 3.36	ethofumesate	1.12, 2.24
alachlor	1.68, 2.24	ethafluralin	1.12, 2.24	desmedipham	0.56, 0.84
alachlor + chloramben	1.68 + 1.68 2.24 + 1.68	chloramben	1.68, 2.24	phenmedipham	0.56, 0.84
dinitramine	0.24 + 0.56	alachlor + chloramben	1.68 + 1.68 2.24 + 1.68	desmedipham + phenmedipham	0.34 + 0.34 0.56 + 0.56

Table 3. Germination response from seeds of two black nightshade sources to no pretreatment and 24 h soak.[a]

Seed source	Seed treatment	
	none	24 h soak[b]
	(% germination)	
Michigan	5 a	37 a
California	18 a	62 b

[a]Means followed by similar letters within columns are not significantly different at the 5% level by Duncan's multiple range test.
[b]Values are the means of treatments.

Table 4. Germination response of seed from two black nightshade sources to running water bath (11 C) over time.[a]

Time in water bath (h)	Seed source MI	Seed source CA
	(% germination)	
1	76 d	68 cd
2.5	52 bc	81 d
5	60 cd	68 cd
7.5	72 cd	33 ab
12	62 cd	32 ab
48	51 bc	38 ab
108	52 bc	22 a

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

Table 5. Growth response to temperature of black nightshade seed from two sources with respect to percent germination, plant height, and dry weight/plant.[a]

Temperature (C)	Germination MI	(%) CA	Plant height MI	(mm) CA	Dry weight/plant MI	(mg) CA
15	0 a	49 cd	0 a	15 ab	0 a	12 a
20	10 b	58 d	19 abc	19 abc	7 a	23 a
25	31 bc	56 d	18 abc	36 bc	26 a	33 a
30	25 b	47 cd	35 bc	38 c	12 a	28 a

[a]Means followed by similar letter within parameters are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Effect of preplant incorporated herbicide application in black nightshade plants from two seed sources with respect to plant number, plant height, and dry weight/plant (expressed as % of control).[a]

Herbicide	Rate (kg/ha)	Plant number Source MI	Plant number Source CA	Plant height Source MI	Plant height Source CA	Dry weight/plant Source MI	Dry weight/plant Source CA
Ethafluralin	1.12	124 efg	116 d-g	76 c	50 abc	42 a	46 a
	2.24	108 d-g	106 d-g	59 bc	26 ab	17 a	16 a
Trifluralin	0.84	103 d-g	106 d-g	145 d	132 d	168 cd	178 cd
Alachlor	1.68	12 a	83 cde	15 a	42 abc	1 a	37 a
	2.24	24 ab	50 bc	24 ab	27 ab	6 a	19 a
Alachlor + chloramben	1.68+1.68	10 a	77 cd	7 a	45 abc	1 a	31 a
	2.24+1.68	10 a	84 cde	12 a	33 ab	3 a	24 a
Dinitramine	0.42	89 c-f	128 fg	145 d	145 d	200 d	155 bcd
	0.56	140 g	113 d-g	129 d	135 d	103 b	149 bcd
Chloramben	1.68	32 ab	100 d-g	29 ab	140 d	7 a	157 bcd
	2.0	27 ab	92 c-f	26 ab	114 d	25 a	130 bc

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

Table 7. Effect of preemergence herbicide application on black nightshade plants from two seed sources with respect to plant number, plant height, and dry weight/plant (expressed as % of control).[a]

Herbicide	Rate (kg/ha)	Plant number Source MI	Plant number Source CA	Plant height Source MI	Plant height Source CA	Dry weight/plant Source MI	Dry weight/plant Source CA
Alachlor	1.68	0 a	27 b-e	0 a	26 abc	0 a	13 a
	2.24	0 a	8 abc	0 a	18 ab	0 a	8 a
Metolachlor	2.24	4 a	41 e	3 a	46 c	1 a	21 a
	3.36	0 a	15 a-d	0 a	46 c	0 a	31 a
Ethalfluralin	1.12	7 ab	34 de	3 a	72 d	1 a	100 b
	2.24	2 a	28 b-e	9 ab	21 abc	8 a	13 a
Chloramben	1.68	5 a	21 a-d	7 ab	33 bc	2 a	31 a
	2.24	7 ab	19 a-d	7 ab	29 abc	1 a	10 a
Alachlor + chloramben	1.68+1.68	0 a	0 a	0 a	0 a	0 a	0 a
	2.24+1.68	0 a	0 a	0 a	0 a	0 a	0 a

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

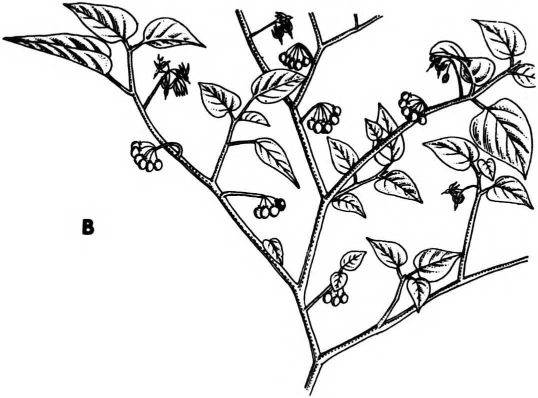
Table 8. Effect of postemergence herbicide application on black nightshade plants from two seed sources with respect to plant height, and dry weight/plant (expressed as % of control).[a]

Herbicide	Rate (kg/ha)	Plant height Source MI	Plant height Source CA	Dry weight/plant Source MI	Dry weight/plant Source CA
Bentazon	0.56	73 d	38 bc	60 f	22 a-d
	1.12	73 d	43 bc	49 ef	24 a-d
Ethofumesate	1.12	58 cd	47 c	28 a-e	29 a-e
	2.24	52 cd	47 c	31 b-e	33 cde
Desmedipham	0.56	52 cd	56 cd	32 cde	27 a-d
	0.84	45 c	36 bc	37 de	16 a-d
Phenmedipham	0.56	53 cd	47 c	29 a-e	11 ab
	0.84	42 bc	12 a	18 a-d	8 a
Desmedipham + phenmedipham	0.34+0.34	48 c	42 bc	28 a-e	12 abc
	0.56+0.56	41 bc	20 ab	27 a-d	10 ab

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

Figure 1. Morphology of Solanum nigrum L. as depicted in Weeds of the North Central States (A) and World's Worst Weeds (B).

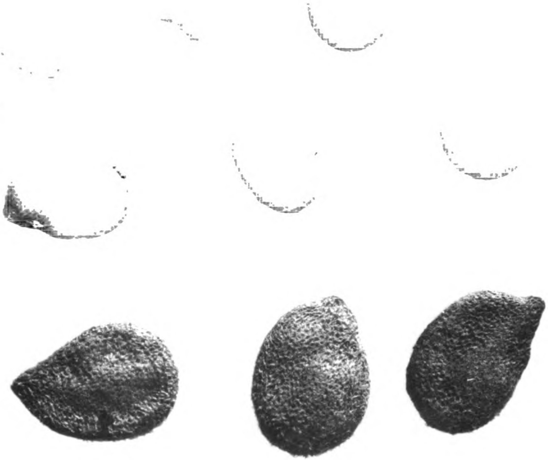
A



B

Figure 2. Seeds of black nightshade obtained in Michigan (A) and from California (B).

A



B

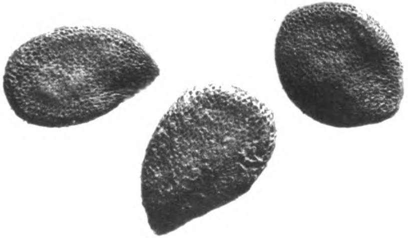


Figure 3. Seedlings grown from black nightshade seed collected in Michigan and obtained from California.


CAL
MICH

Figure 4. Mature leaves from plants grown from seed collected in Michigan (A) and obtained from California (B).

A

B

Figure 5. Flowers on plants grown from black nightshade seed collected in Michigan (A) and obtained from California (B).

A

B

CHAPTER 3

ABSORPTION, TRANSLOCATION, AND METABOLISM OF ETHAFLURALIN AND TRIFLURALIN IN *SOLANUM* *spp*.

ABSTRACT

Roots of black nightshade (*Solanum* *sp*.) seedlings treated with ^{14}C-ethalfluralin (*N*-ethyl-*N*-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) and ^{14}C-trifluralin (a,a,a-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) were grown to determine the basis for differences in response by plants from two different seed sources.

Plants grown from the California seed source absorbed more ^{14}C-ethalfluralin and ^{14}C-trifluralin than plants from the Michigan seed sources although no differences were seen in susceptibility between plants grown from the seed sources subjected to preplant incorporated herbicide application in earlier research. During the first 24 h, seedlings from the California seed source absorbed more ^{14}C-ethafluralin than did plants treated with ^{14}C-trifluralin. After absorbed of ^{14}C-ethalfluralin and ^{14}C-trifluralin were equal by plants from the Michigan seed source.

More ^{14}C-ethalfluralin than ^{14}C-trifluralin was found in the black nightshade shoots of both seed sources. The activity of ^{14}C in roots was similar after treatments with the two herbicides which could indicate a greater activity of ethalfluralin.

Seventy-two h after treatment with ^{14}C-labelled herbicides, the

conversion to the water-soluble fraction was greater for ^{14}C-ethalfluralin than for ^{14}C-trifluralin. In the shoots of both seed sources an average ^{14}C-concentration of nearly 55% occurred in the water-soluble fraction following ^{14}C-ethalfluralin treatment while an average of only 40% was in the water-soluble fraction following ^{14}C-trifluralin treatment.

INTRODUCTION

Early studies (4) showed very little absorption and translocation of trifluralin (a,a,a-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) incidating that translocation was not sufficient to be of any consequence (8). Probst *et al*. (12) did not detect trifluralin or any of its degradation products in the leaves, seeds, or fruits of soybean (*Glycine max* L. Merr.) and cotton (*Gossypium hirsutum* L.). However, trifluralin was found in the outer layers of root crops such as onion (*Allium sp*. L.) and garlic (*Allium sativum* L.). This suggested that the incorporation of the ^{14}C-trifluralin based on the universal distribution of the radioactivity without definite parent or metabolite identification. Kechersid *et al*. (7) observed acropetal and basipetal trifluralin movement in peanut (*Arachis hypogala* L.). ^{14}Carbon labelled C-trifluralin was found in all parts of three stages of peanut seedlings (leaves, epicotyl, cotyledons, hypocotyl, and roots), indicating extensive translocation. Penner (10) showed that temperature at which plants are grown had a greater effect than temperature at the time of treatment. He also found ^{14}C-trifluralin in roots and shoots of corn (*Zea Mays* L.) and soybean. Generally, more ^{14}C was found in roots and shoots of corn than soybean. Hawby (5) found barnyardgrass (*Echinochloe crus-galli* (L.) Beauv.) and sorghum (*Sorghum* bicolor (L.) Moench), the susceptible species, accumulated more ^{14}C-profluralin (N-(cyclopropylmethyl)-a,a,a-trifluoro-2,6-dinitro-N-propyl-p-toluidine) and ^{14}C-dinitramine (N^{4},N^{4}-diethyl-a,a,a-trifluoro-3,5-dinitrololuene-2,4-diamine) in roots and shoots than did resistant species Palmer amaranth (Amaranthus palmeri S. Wats) and soybean. Little ^{14}C-profluralin translocated to the shoots, whereas much ^{14}C-dinitramine accumulated in the plant shoots.

Considering the species in which C absorption and translocation have been found (e.g., cotton and soybean (14), sorghum (1), sweet potato (Ipomea butatas L.) (2), red kidney bean (Phaseolus vulgaris L.) (3) and the previously mentioned species) it appears that substituted dinitroaniline absorption and translocation is herbicide dependent and that more ^{14}C translocation is seen in susceptible than tolerant species.

Recently, researchers (15,16) have found that two Solanum sp. gave different responses to ethalfluralin (N-ethyl-N(2-methyl-2-propenyl)-2, 6-dinitro-4-(trifluoromethyl)benzenamine) and trifluralin. Skylakakis et al. (13) reported that ethalfluralin application resulted in at least 86% control of black nightshade (Solanum nigrum L.). Koren et al. (9) reported ethalfluralin application controlled annual grasses and broad-leaved weeds in cotton, even trifluralin resistant Solanum sp.

The objectives of this study were to determine whether these previously observed differences between ethalfluralin and trifluralin could be explained on the basis of absorption, translocation, or metabolism.

MATERIALS AND METHODS

Native black nightshade (seed source I) fruit was collected from Sandusky, MI, after falling from the mature plant, allowed to dry, and cleaned. Seed for the second Solanum sp. was secured from a California based commercial seed supplier (Valley Seed Co., Fresno). Seed from both sources was stored at 11 C until 48 h prior to use and then at room temperature.

Seed from both sources was germinated and grown in #18 Ottawa washed quartz sand in 50% Hoagland's nutrient solution (6) in growth chambers with 16 k/day at 25 $\pm$ 2 C. Light intensity at plant level was 24 to 27 klux provided by a mixture of fluorescent and incadescent lamps. At the two to four leaf stage, seedlings of both sources were transplanted to foil wrapped 21 by 70 mm glass shell vials with sponge supports containing 50% Hoagland's solution. Uniformly ring labelled ^{14}C-ethalfluralin and ^{14}C-trifluralin were used with specific activities of 2.78 µci/umole and 2.53 µci/umole, respectively. Stock solutions were made by dissolving each herbicide in methanol.

After 24 to 42 h acclimatization period, the most uniform seedlings were selected. The remaining untreated nutrient solution was discarded and 14 ml 0.33 x 10^{-6}M ^{14}C-ethalfluralin or 0.33 x 10^{-6}M ^{14}C-trifluralin in 50% Hoagland's solution was added to each vial. Three plants, one per vial, were used per treatment. Twenty-four and 72 h exposure times were used. Two plants from each treatment were saved for autoradiography. The remaining plants were pooled for quantitative ^{14}C determination described below.

At harvest, nutrient solution uptake by each plant was determined.

Plant roots were rinsed three times in distilled water and blotted dry. Plants were placed on dry ice, freeze dried, and then autoradiographed or divided into shoot and root for dry weight determination and extraction.

Plant parts were homogenized in 20 ml methanol in a Sorvall Omni-Mixer at high speed for 5 min. Plant homogenates were filtered through Whatman No. 4 filter paper and rinsed with methanol. The methanol-insoluble residue on Whatman No. 4 filter paper was freeze-dried prior to combustion.

The methanol-insoluble residue was combusted by placing the sample in a platinum basket into a 1000 ml combustion flask and purging the flask with oxygen for 1 min. After samples were ignited in a Nuclear Chicago Model 3151 semi-automatic combustion system, $^{14}CO_2$ was absorbed in 15 ml absolute ethanol plus ethanol-amine (95%) (2:1 v/v), injected through a rubber septum stopper and stirred for 15 min. Aliquots (1 ml) of the $^{14}CO_2$ absorbant was radioassayed by liquid scintillation spectrometry using a 15 ml ACS^R aqueous scintillation solution. Dissintegrations per minute were calculated correcting for combustion efficiency.

The methanol-soluble extract was concentrated en vacuo to 1 ml. This fraction was partitioned once with 10 ml of 9:1 (v/v) hexane:water. The aqueous layer was partitioned three additional times with 10 ml hexane. These four hexane fractions were pooled and the volume reduced under vacuum to 1 ml. A 100 µl sample of the water-soluble fraction was radioassayed using 4 g PPO (2,5-diphenyloxazole) plus 50 mg dimethyl POPOP (1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene) in 333 ml TRITON X-100 plus toluene and brought to a volumn of one 1. Samples (10 µl) of the hexane-soluble fraction were radioassayed using the PPO plus POPOP scintillation solution used for quantitative ^{14}C-ethalfluralin and

^{14}C-trifluralin determination. The second 10 µl sample was spotted on 250 µm Silica Gel GF thin-layer chromatography plates. All plates included standards for purity and parent herbicide identification. Thin-layer plates were subjected to radioautography for 2 weeks and developed. All data presented are the means of two experiments.

RESULTS AND DISCUSSION

Black nightshade plants from the CA seed source absorbed more ^{14}C-ethalfluralin and ^{14}C-trifluralin, 24 and 72 h after treatment, than plants from the MI source (Table 1). Considering plants from a single seed source there was no difference in absorption between the two herbicides. Plants grown from both black nightshade seed source, MI and CA, have responded equally to preplant incorporated but not preemergence ethalfluralin and trifluralin chemical treatments (15,16). Plants from the Michigan seed source have been more susceptible to treatment by ethalfluralin than trifluralin. Since absorption of the two herbicides is similar, absorption does not appear to play a role in the differential response to these two herbidides.

The ^{14}C-distribution patterns for ethalfluralin and trifluralin resulted in significant herbicide by plant part, seed source by plant part, and application time by plant part interactions (Table 2, Figures 1 and 2). More ^{14}C-ethalfluralin accumulated in the shoot regardless of treatment than did ^{14}C-trifluralin whereas the same concentration was found in the root. The plants grown from the CA seed source accumulated between three and four times more ^{14}C-herbicide in both the shoot and root than did the plants from the MI seed source. More ^{14}C-herbicide was found in the root than in the shoot regardless of plant seed source or exposure time. However, more ^{14}C was found in the root after 24 h than 72 h. These data indicate that when discussion plants grown from different seed sources, type of herbicide, or exposure time; the plant parts must be considered. Plants grown from the MI seed source accumulated less ^{14}C than did those from the CA seed source, in both the shoot and root.

Based on the metabolite separation obtained using the various extraction and TLC systems used by past researchers (2,4,5) we decided to utilize the extraction procedure and carbon tetrachloride used twice TLC development system of Golab (4). The hexane-soluble fraction of the extraction procedure was subjected to TLC analysis for potential metabolism detection due to the low amounts of radioactivity in the chloroform and aqueous extractant phases found by Golab (4).

Analysis of the percent disintegrations per minute per milligram dry plant material found in the hexane and methanol-insoluble fractions resulted in significant exposure time by seed source by plant part interactions (Table 3). Data for the water-soluble fraction resulted in significant herbicide effect and seed source by plant part interaction. Plants from the CA and MI source accumulated less ^{14}C in almost every case in the shoot than in the root regardless of exposure time. There was an increase in ^{14}C found in the water-soluble fraction and in most cases less ^{14}C in the hexane-soluble fraction in the shoot as exposure time progressed. Significantly more methanol-insoluble ^{14}C-material was found in the shoots of the plants grown from the MI seed source with 24 h exposure than any other treatment. There was more ^{14}C in the methanol-insoluble fraction as exposure time increased in the root portion of plants grown from the CA seed source and shoots of plants from the MI seed source. Thin-layer chromatography of the hexane-soluble fraction indicated the presence of only the parent herbicides. Twenty-four hours after treatment over 25% of the ^{14}C in the shoots was in the water-soluble fraction (Table 3). This concentration in the water-soluble fraction increased with time. Ethalfluralin metabolism was more rapid in the black nightshade from the CA seed source than from the MI seed source. Ethalfluralin itself was converted more rapidly into water-soluble metabolites than trifluralin; this difference was very evident in the roots where up to six-fold differences were found indicating that the double bond in the carbon chain of ethalfluralin may be more reactive than the alkyl moiety of trifluralin.

Extraction procedures using parent compounds alone were examined. The hexane and water fractions were radioassayed by liquid scintillation spectrometry and over 98% of the total radioactivity was found in the hexane fraction. This indicated that the ^{14}C found in the water fraction must be metabolites for both ethafluralin and trifluralin. These results are consistent with those of Penner and Early (11) indicating rapid metabolism of trifluralin into the water-soluble fraction of corn.

The increased absorption and translocation seen from ethalfluralin relative to trifluralin to the shoot may be the reason for increased black nightshade efficacy which has resulted from ethalfluralin in the field. The rapid incorporation into the water fraction seen in plants from the CA seed source regardless of herbicide or exposure may be the reason why even though larger amounts were absorbed, the same response was seen from plants of both seed sources.

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Table 1. Absorption of ^{14}C-ethafluralin and ^{14}C-trifluralin by plants from two black nightshade seed sources grown in nutrient culture after 24 h and 74 h exposure.[ab]

Seed source	Ethalfluralin		Trifluralin	
	24 h	72 h	24 h	72 h
Michigan	2449 ab	1771 ab	3012 b	1661 a
California	7792 d	4809 c	5656 c	4655 c

[a]Values are expressed as total dpm/plant.
[b]Means followed by common letters are not significantly different by Duncan's multiple range test.

Table 2. Distribution of ^{14}C-ethalfluralin and ^{14}C-trifluralin in plants grown from two black nightshade seed sources in shoot and root over 24 and 72 h exposure.[ab]

	Plant parts	
	Shoot	Root
^{14}C-herbicide		
Ethalfluralin	575 b	1329 c
Trifluralin	182 a	1443 c
Seed source		
Michigan	231 a	854 b
California	526 c	1918 d
Exposure period (h)		
24	367 a	1778 c
72	389 a	955 b

[a]Values are expressed as dpm/plant.
[b]Means within treatment followed by common letters are not significantly different by Duncan's multiple range test.

Table 3. Metabolites of ^{14}C-ethalfluralin and ^{14}C-trifluralin in shoot and root components of plants grown from two black nightshade seed sources after 24 and 72 h exposure.[ab]

			Exposure time					
			24 h			72 h		
Herbicide	Seed Source	Plant part	Water fraction	Hexane fraction	Methanol insoluble fraction	Water fraction	Hexane fraction	Methanol insoluble fraction
Ethalfluralin	Michigan	shoot	27.0	66.5 c-f	6.2 a-d	45.0	44.2 ab	11.0 cd
		root	12.5	81.0 fgh	6.5 a-d	21.8	72.0 efg	9.0 bcd
	California	shoot	48.0	49.2 bc	2.8 ab	64.2	29.2 a	6.8 a-d
		root	25.2	71.8 efg	2.5 ab	36.2	51.5 bcd	12.8 d
Trifluralin	Michigan	shoot	29.5	43.5 ab	27.0 e	35.5	56.0 b-e	8.8 a-d
		root	2.8	96.0 h	1.2 a	5.8	89.2 gh	4.5 abc
	California	shoot	27.0	69.2 def	9.0 bcd	45.5	45.0 ab	9.5 bcd
		root	2.8	95.2 h	3.0 ab	6.0	90.2 gh	3.8 abc

Seed source	Water fraction plant part: shoot	Water fraction plant part: root
Michigan	34 c	11 a
California	46 d	18 b

Chemical	%dpm/mg
Ethalfluralin	35 b
Trifluralin	19 a

[a]Values are expressed as percent of total dpm/mg recovered.
[b]Means within each fraction having common letters are not significantly different by Duncan's multiple range test.

Figure 1. Distribution of ^{14}C-ethafluralin in plants grown from seed collected in Michigan (A) and obtained from California (B). The treated plant specimen is above the corresponding autoradiograph.

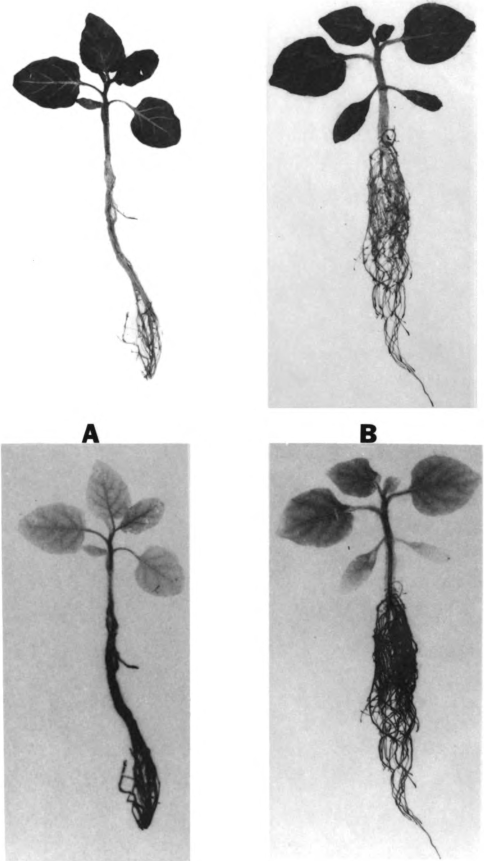
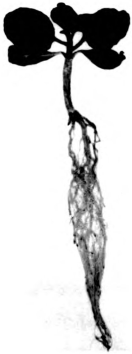
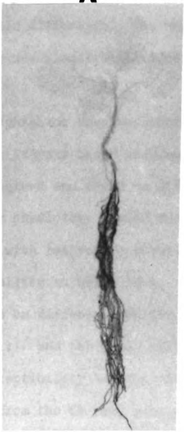

A
B

Figure 2. Distribution of ^{14}C-trifluralin in plants grown from seed collected in Michigan (A) and obtained from California (B). The treated plant specimen is above the corresponding autoradiograph.



A **B**



CHAPTER 4

SUMMARY AND CONCLUSIONS

Field studies were conducted to evaluate efficacy of several herbicides for black nightshade control, activity on navy beans as a primary crop and wheat as a rotational crop, and soil persistence. In general, alachlor was most efficacious for black nightshade control while little difference among herbicides was seen with respect to navy bean yield and no differences among herbicides tested were seen on wheat stand or yield. The application of chloramben or dinoseb either tank mixed and preplant incorporated or preemergence resulted in no general navy bean yield difference. The weed control obtained from chloramben or dinoseb as additional herbicides often increased black nightshade control.

Black nightshade seed was obtained from two sources, one native and one from a California based seed supplier. Plants from these two seed sources were grown and found to differ morphologically. Further examination of their physiology showed differences between plants from the two seed sources with respect to germination, growth response to temperature, and susceptibility to herbicides. Plants from these two seed sources were found to be different species entirely, one was (MI) *Solanum americanum* Mill. and the other (CA) was *Solanum scabrum* Mill. Both differed in susceptibility to ethalfluralin and trifluralin.

Plants from the CA seed source absorbed more ^{14}C-ethalfluralin and

^{14}C-trifluralin than the plants from MI. Distribution of the ^{14}C-herbicides resulted in more ^{14}C-ethalfluralin than ^{14}C-trifluralin in the shoots and the same concentrations of each in the roots. Twenty-four hours after treatment metabolism was more rapid for ^{14}C-ethafluralin especially in plants from the CA seed source as indicated by the greater concentration of ^{14}C detected in the water-soluble fraction.

Differences in activity of these two substituted dinitroanilines on black nightshade may be due to differing rates of herbicide metabolism. Absorption did not appear responsible for differences in activity.

Weed researchers commonly order readily available seed for experimentation, field and laboratory. Rarely in the literature has the weed seed source been listed. This practice perpetuates thoughts that seed collected near the researchers locale was used. The seed source must be given or improper assumptions will continue. Herbicide labels are written and often applicable nationwide whereas what is called by similar names in dissimilar localities may cause incorrect herbicide usage by farmers and researchers alike.

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