

TO 2-CHLORO-2',6'
(METHOXYMETHYL) ACETA

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Factors affecting the response of yellow
nutsedge (Cyperus esculentus L.) to 2-chloro-
2',6'-diethyl-N-(methoxymethyl) acetanilide
(alachlor)

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Thomas F. Armstrong

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ABSTRACT

FACTORS AFFECTING THE RESPONSE OF YELLOW NUTSEDGE (CYPERUS ESCULENTUS L.) TO 2-CHLORO-2',6'-DIETHYL-N-(METHOXYMETHYL) ACETANILIDE (ALACHLOR)

By

Thomas F. Armstrong

Preplant or preemergence applications of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) at 3.36 or 4.48 kg/ha effectively controlled yellow nutsedge (Cyperus esculentus L.) in field and greenhouse studies. Depth of tubers in the soil did not affect nutsedge response to alachlor. Alachlor at 3.36 kg/ha did not effectively control yellow nutsedge in soils with more than 6% organic matter. Alachlor at 3.7×10^{-6} M and 18.5×10^{-5} M inhibited growth and killed newly emerging shoots in Petri dish cultures. However, alachlor did not inhibit sprouting of yellow nutsedge tubers.

The main site of uptake of alachlor by small yellow nutsedge plants is the tissue above the tuber. Primarily acropetal and some basipetal ^{14}C -translocation occurred from ^{14}C -alachlor applications to small seedlings with 2 to 4 cm shoots. Alachlor is metabolized rapidly in yellow nutsedge to at least one water-soluble metabolite. Applications of ^{14}C -alachlor to 15 cm shoots resulted in limited acropetal ^{14}C -translocation.

FACTORS AFFECTING THE RESPONSE OF YELLOW NUTSEDGE (CYPERUS
ESCULENTUS L.) TO 2-CHLORO-2',6'-DIETHYL-N-
(METHOXYMETHYL) ACETANILIDE (ALACHLOR)

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Thomas Fred Armstrong

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INTRODUCTION

Man has identified, propagated and used many plants. Some of these plants are pests in lawns, gardens and agricultural fields; one of these plants, yellow nutsedge (Cyperus esculentus L.) has become a serious weed problem. Due to the severity of the nutsedge problem, an economical control program is needed. Therefore, when a new herbicide is developed, it should be applied to nutsedge to determine any toxic effects and develop control methods.

A new herbicide, 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide (alachlor), for use in corn (Zea mays L.) and soybeans [Glycine max (L.) Merr.] was reported to be phytotoxic to yellow nutsedge (1, 10, 13, 22, 24). The purpose of this study was to more fully understand the factors affecting the efficacy of alachlor on yellow nutsedge in field, laboratory, and greenhouse studies.

CHAPTER 1

Yellow nutsedge (Cyperus esculentus L.)

Both purple (Cyperus rotundus L.) and yellow nutsedge (Cyperus esculentus L.) cause important weed problems in crops (47, 48, 50, 86). Purple nutsedge has been ranked as the most important single weed in the developing countries of the world (47, 48). Yellow nutsedge is found in most states of the United States (65, 85, 86), and its infestation into cropland is increasing (25, 60, 85).

Yellow nutsedge is a perennial sedge with numerous slender rhizomes ending in tubers or shoots (36). The morphological characteristics of the plant are affected by the length of the photoperiod (9, 17, 55). Maximum shoot and tuber formation from indeterminate rhizome tips occurs with photoperiods of 16 hr and 8 to 12 hr (55). Flowering occurs between photoperiods of 12 to 14 hr. Tubers are also produced by mature plants under long day conditions (9).

In yellow nutsedge tubers, the buds are located at the apical end, arranged in sets of three, and positioned as at points of an equilateral triangle (13). Most tubers contain two sets of three buds, the second set is inside the triangle formed by the first set (13). The largest bud of the outer set sprouts first, and the destiny of sprouted buds determines the number of buds which ultimately sprout

from a given tuber (13). The vascular tissue of rhizomes, which terminate in tubers, extends through the tubers and into the buds, roots, and rudimentary leaves of the new shoot (13).

Both viable seeds and tubers are produced by yellow nutsedge (9, 10, 45, 84). In one growing season, a single seedling developed into a stand of plants that produced a yield of 90,000 seeds of which 51 percent germinated (45). One tuber in the field produced 1900 plants plus 6900 tubers in an area 3.2 m² in 1 year (84). Eight-five percent of the tubers are found in the top 15.2 cm of soil. Each plant produces seven to nine tubers, borne singly, on terminal rhizomes. A tuber produces up to seven shoots, but a given shoot produces many rhizomes that give rise to new shoots or tubers (84). These tubers have the capacity for at least three repeated sproutings (80). Over 60% of the tuber dry weight, carbohydrate, oil, starch, and protein are consumed during the first sprouting, but less than 10 percent of these materials are utilized during each of the remaining two sproutings. Tubers, may be significantly different in weight, but have the same sprouting percentage (80).

Tuber sprouting is affected by depth of planting in the soil, soil compaction, dormancy, and mechanical disturbance. In the soil, tubers sprout readily at 7.6 to 15.2 cm depths; below this soil level, and in compacted soils sprouting is reduced (17). Even though sprouting is reduced at lower soil

depths, shoots will grow from tubers 30.5 cm deep in the field and tubers 50.8 cm to 81.3 cm deep in the greenhouse (84). In newly formed tubers, the percent sprouting is very low (9, 17, 82). This dormancy can be broken with low temperatures, chemicals, or by washing in 13 C water (9, 17, 58, 84). Both mowing and disk-harrow cultivation of a yellow nutsedge stand significantly increased tuber sprouting during a period when sprouting from tubers in an undisturbed stand was low (82). Tillage operations that exposed tubers on the soil surface for 2 days lowered sprouting percentage 80 percent (84). Tubers that were exposed to 4 C had a lower survival than tubers exposed to 22 C. Duration of dessication did not influence tuber survival, except at the 4 C temperature (83).

Yellow nutsedge is a persistent pest in many of our agricultural crops in the United States and the world. Its infestation into agricultural areas is increasing (85).

Biological control of yellow nutsedge

The insect, Bactra verutana Zeller (Olethreutidae), has been observed to attack and destroy yellow nutsedge plants in California. Specific plant injury symptoms were dessication of the intermost leaves and severe injury to growing points. This insect is limited in its usefulness as a biological control agent because of spring emergence of yellow nutsedge and late summer occurrence of Bactra verutana Zeller, the prolific vegetative nature of yellow nutsedge, and insect parasites on Bactra verutana Zeller (62).

Mechanical control of yellow nutsedge

In an attempt to eradicate yellow nutsedge, workers at Cornell University kept nutsedge infested land fallow for 4 years (9). After four years, 119 tubers/m² were found in the top 15 cm of soil compared to 5821 tubers/m² in the check. These tubers sprouted and eventually new tubers were formed. Therefore, mechanical methods reduced the severity of the nutsedge problem but did not eliminate it.

Chemical control of yellow nutsedge

Chemical control offers a means of controlling yellow nutsedge. Various herbicides have been applied to yellow nutsedge, but few effectively control this sedge.

Arsenical herbicides have been applied to yellow nutsedge in turf. Tubers collected from plants treated with 3.36 kg/ha of disodium methanearsonate (DSMA) or monosodium methanearsonate (MSMA) sprouted in significantly reduced numbers compared to control tubers (61). Small tubers contained a significantly higher concentration of arsenic (23 to 33 ppm) than large tubers (4 to 12 ppm) and the herbicides reduced the vitality of small tubers but had little or no effect on vitality of large tubers (61). DSMA and MSMA have provided 80% or greater control of yellow nutsedge (28, 59), but MSMA caused injury to the turf (28).

In New York State, sodium 2,2-dichloropropionate (dalapon) applied to yellow nutsedge plants 10.2 cm in height gave complete control in two of four locations (77). Investigations using ^{14}C -dalapon indicated there was negligible basipetal movement of ^{14}C after leaf absorption, but there was excellent distribution after root absorption (77). In the previous ^{14}C -dalapon experiments only small amounts of ^{14}C accumulated in the parent tuber of the plant (77), and dalapon had little effect on the viability of intact tubers (22).

In potatoes treated with 5.6 kg/ha of dalapon in 1 and 2 applications, the dry weight of yellow nutsedge was reduced considerably, however the nutsedge was not dead (6). The highest yield of potatoes was obtained in the aforementioned experiment with 28 kg/ha of the sodium salt of trichloroacetic acid (NaTCA) which gave some control of yellow nutsedge.

Yellow nutsedge plants treated with 3-amino-1,2,4-triazole (amitrole) produced tubers containing amitrole. These tubers rarely sprouted and the seeds had lower germination (9). Radioautographs of yellow nutsedge treated with ^{14}C -amitrole, indicated translocation from the point of application to the seed (45). Both acropetal and basipetal translocation was observed in 30.5 cm high yellow nutsedge plants. Amitrole applied to the foliage translocated to the intact tubers and greatly reduced their viability (22).

EPTC (S-ethyl dipropylthiocarbamate) has been used to control yellow nutsedge in corn and potatoes (34, 52, 74). Other thiocarbamates, butylate (S-ethyl diisobutylthiocarbamate) and vernolate (S-propyl dipropylthiocarbamate) have been used in corn and soybeans, respectively (34, 87). Excellent yellow nutsedge control was obtained with 4.5 to 6.7 kg/ha of EPTC and butylate (29, 34); these two herbicides provided greater yellow nutsedge control if applied during or after germination (23). In Illinois, vernolate equalled EPTC for yellow nutsedge control and resulted in less injury to soybeans (87).

Yellow nutsedge tubers germinated readily in soil treated with EPTC. However, shoot growth was suppressed and emergence delayed, the delay depended on the rate of application (49). The placement of EPTC in the soil affected the control of yellow nutsedge (43). As the depth of EPTC placement in the soil increased, yellow nutsedge control decreased (44). Butylate placed in the tuber zone effectively suppressed shoot growth and tuber sprouting, but it was ineffective when incorporated above or below the tubers (53). Apparently the thiocarbamates need to be placed near the yellow nutsedge tubers to give control.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] has been applied in a preplant, preemergence and post-emergence manner to yellow nutsedge (21, 23, 29, 34, 73).

Preplant incorporated applications of atrazine at 2.2 to 4.5 kg/ha applied during or after tuber sprouting controlled nutsedge (23). Two cultivations following preemergence applications of atrazine during a dry season significantly reduced yellow nutsedge stands compared to the use of atrazine with cultivation (63). Preemergence applications of atrazine require more rainfall for activation (34). The addition of non-phytotoxic oil to early postemergence applications of atrazine increased nutsedge control (21, 23).

Atrazine injured new plants from germinating tubers but did not kill dormant tubers (9). Translocation studies with ^{14}C -atrazine, showed that atrazine does not accumulate in the tubers but is readily translocated throughout the nutsedge plant (8). This explains why incorporated applications of atrazine were less effective when tubers were dormant than at later stages (23).

2,4-D (2,4-dichlorophenoxy acetic acid) has been used for nutsedge control with varying degrees of success. Bhan et al. (15) reported that 2,4-D significantly reduced shoot weight, rhizome weight, and rhizome number 12 days after application (15). In turf, repeated application of 2,4-D significantly reduced the number of yellow nutsedge shoots per square foot (28). Other researchers have found that 2,4-D did not control this sedge (6, 53, 69).

Various substituted urea herbicides have also been tried for yellow nutsedge control (7, 34, 78). Monuron

3-(p-chlorophenyl)-1,1-dimethylurea applied at 11.2 kg/ha significantly reduced the number of yellow nutsedge plants (6). Preemergence applications of monuron at 5.6 kg/ha reduced top growth by as much as 63% and tuber production by 98% (7). Preemergence or postemergence applications of 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) at 2.24 kg/ha did not control yellow nutsedge (34).

Alachlor and yellow nutsedge

During 1969, a new herbicide 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide alachlor registered for weed control in soybeans [Glycine max (L.) Merr.] and corn (Zea mays L.) (88) was reported to be phytotoxic to yellow nutsedge (2, 37, 58, 87, 90). Yellow nutsedge control in corn was obtained with preplant incorporated or preemergence applications of alachlor (2, 14, 37); and in soybeans 90 percent control resulted from preplant incorporation treatments (87). Under dry conditions, preemergence treatments of alachlor gave only fair control (27, 87), but a shallow incorporation of not more than 5 cm provided equal or better control (27, 87).

Alachlor placed near yellow nutsedge tubers in soil reduced shoot growth (53, 87). Worthington (90) has shown that alachlor delayed the emergence of yellow nutsedge plants and increased the number of tuber rhizomes that

developed from each tuber. Godke reported significant reduction of tuber production with preemergence applications of alachlor (37).

The mode of action of alachlor is unknown. Since alachlor's chemical structure is similar to N-isopropyl-2-chloroacetanilide (propachlor), it may affect protein synthesis (26, 57). Possibly nitrate reductase is inhibited by alachlor (21), or the activation of amino acids is prevented (57). Alachlor was found to have no detrimental effect on soil microbial activity at recommended field rates (67).

CHAPTER 2

Growth suppression of Yellow Nutsedge with Alachlor

Abstract

Preplant or preemergence application of 2-chloro-2', 6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) at 3.36 or 4.48 kg/ha effectively controlled yellow nutsedge (Cyperus esculentus L.) in field and greenhouse studies. Depth of tubers in the soil did not affect nutsedge response to alachlor. Alachlor at 3.36 kg/ha did not effectively control yellow nutsedge in soils with more than 6% organic matter. Alachlor at 3.7×10^{-6} M and 18.5×10^{-5} M inhibited growth and killed newly emerging shoots in Petri dish cultures. However, alachlor did not inhibit sprouting of yellow nutsedge tubers.

Introduction

Yellow nutsedge is increasing as a weed problem in most states of the United States (7, 13, 14, 18). Both viable seeds and tubers are produced by yellow nutsedge (2, 3, 10, 17), but propagation by tubers is the most important means of dissemination in cultivated crops (2). One tuber in a Minnesota field produced 1900 plants plus 6900 tubers in a 3.2 m² area in 1 year (17).

Tuber sprouting is affected by tuber depth in soil, soil compaction, tuber dormancy, and mechanical disturbance. In the soil, tubers sprout readily at 7.6 to 15.2 cm depths; below this soil level and in compacted soil, sprouting is reduced (4). The percent sprouting is very low from newly formed tubers (2, 4, 16). Both mowing and disk-harrow cultivation of a yellow nutsedge stand significantly increase tuber sprouting (16).

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] has been applied in a preplant, preemergence and postemergence manner for yellow nutsedge control (5, 6, 8, 9, 15). Preplant incorporated applications of atrazine at 2.2 to 4.5 kg/ha controlled yellow nutsedge if applied during or after, rather than before tuber sprouting (6). Preemergence applications of atrazine require rainfall for activation (9). The addition of non-phytotoxic oil to early postemergence applications of atrazine increased nutsedge control (5, 6). Atrazine injured new plants from germinating tubers but did not kill dormant tubers (2).

In 1969, alachlor was registered for weed control in soybeans [Glycine max (L.) Merr.] and corn (Zea mays L.); and reported to be phytotoxic to yellow nutsedge (1, 13, 21).^{1, 2}

¹Godke, D. L. 1969. The control of yellow nutsedge with alachlor and an alachlor plus linuron mixture. M.S. Thesis. Southern Illinois University, Carbondale. 58p.

²Worthington, J. P. 1971. The effect of alachlor and Mon-097 on the growth of yellow nutsedge and the uptake of ¹⁴C-labeled alachlor by yellow nutsedge and soybeans. Ph.D. Thesis. Ohio State University, Columbus. 62p.

Alachlor placed near yellow nutsedge tubers in soil reduced shoot growth (11, 20). Worthington³ has shown that alachlor delayed the emergence of yellow nutsedge plants and increased the number of tuber rhizomes that developed from each tuber. Godke⁴ reported a significant reduction in tuber production with preemergence applications of alachlor.

The objectives of this study were to evaluate the response of yellow nutsedge to alachlor in the field, greenhouse, and laboratory and to develop an effective yellow nutsedge control program with alachlor.

Materials and Methods

Field studies during 1969, 1970 and 1971 were conducted at E. Lansing, Michigan on a Conover sandy loam (2.8% organic matter) with a native infestation of yellow nutsedge. The field was fall plowed, the plots were 3.1 by 10.8 m, and the treatments were replicated three times in a randomized complete block design. The preplant treatments were incorporated twice with a springtooth harrow and Michigan 400 corn was planted. The herbicides were applied with a tractor mounted sprayer at 30 psi and 215 L/ha. The plots were not cultivated, and were evaluated approximately 4 weeks after herbicide

³Ibid.

⁴Godke, D. L. op. cit.

application. Yellow nutsedge control was rated on a 0 to 10 scale where 0 = no control and 10 = complete control or kill. Only the 2 middle rows (152 cm) of each 4-row plot were rated.

Greenhouse studies were conducted to determine yellow nutsedge response to alachlor as affected by depth of tubers in a loam soil. Tubers that received 6 weeks of 7 C cold treatment were sprouted in Petri dishes. Nine sprouted tubers each with less than 1 cm shoot length were planted at either 2.5, 10.2, or 20.3 cm depths in each 30.5 by 33.0 by 43.2 cm wooden box. Water was added as needed throughout the experiment to maintain soil moisture. Preemergence applications of alachlor at either 0.00, 2.24 or 4.48 kg/ha were made for each tuber depth. After 6 weeks, the yellow nutsedge was harvested and dry matter determined. The treatments were replicated three times in a randomized complete block design.

After harvest of the yellow nutsedge, a perennial ryegrass bioassay was used to determine alachlor residue in the loam soil. Soil samples from the previous experiment of 0.0 to 5.1, 5.1 to 10.2, and 10.2 to 15.2 cm depths were placed in a 750 cm³ styrofoam container and 30 ryegrass seeds were planted in each container. After 2 weeks of growth, the ryegrass was harvested and mg fresh weight/plant recorded.

The response of yellow nutsedge to various levels of organic matter and preemergence alachlor treatments was examined. A Conover sandy loam soil with 2.84% organic matter and a muck soil with 81.40% organic matter were mixed together in various volume ratios to obtain 2.84%, 4.57%, 5.55%, 10.28% and 81.40% organic matter soils. One tuber was planted 3 cm deep in each 473 ml container containing the respective level of organic matter. Preemergence applications of alachlor were made at either 0.00, 1.12 or 3.36 kg/ha. Shoot emergence and shoot height were recorded after 8 weeks. Water was added throughout the duration of the experiment to maintain soil moisture. The treatments were replicated seven times in a completely randomized design.

Laboratory studies were used to determine the effect of alachlor on yellow nutsedge tuber sprouting and growth. Tubers were sprouted in Petri dishes containing solutions of 0, 3.7×10^{-6} M, or 18.5×10^{-5} M alachlor. Fifteen tubers were placed in each Petri dish. After 5 days the percent of tubers sprouting and shoot length were recorded. Each treatment was replicated three times in a completely randomized design.

The effect of tuber exposure time to 3.7×10^{-6} M alachlor on yellow nutsedge shoot length was determined by placing tubers in Petri dishes containing solutions of 3.7×10^{-6} M alachlor for 0, 8, 24, 48, 72, 96, 120 and

144 hrs. At the end of each time exposure, the respective tubers were rinsed off six times with distilled water and then replaced in Petri dishes, five per dish, containing only distilled water. Shoot length was recorded at 1, 2, 3, and 4 weeks after the start of experiment. Each treatment was replicated three times in a completely randomized design.

Tubers were sprouted in 0 and 3.7×10^{-6} M alachlor solutions in Petri dishes for 1 week. The sprouted tubers were then transferred to 293 ml plastic cups containing washed sand. The tubers were planted 2 cm deep, and number of shoots present on each tuber and the shoot length were recorded. One half of the alachlor treated tubers received Hoaglands no. 1 nutrient solution with alachlor for 3 weeks. The cups containing the tubers were placed in a growth chamber at 25 C. At the end of the experiment new shoot emergence from the cups and shoot length were recorded. The treatments were replicated four times in a completely randomized design. All data presented is the mean of two experiments with two or more replications per experiment.

Results and Discussion

The amount of rainfall during 10 days after the start of treatment influenced the degree of yellow nutsedge control. The amounts of rainfall during the 10 days after herbicide application for each year were the following: 1969, 3.3 cm; 1970, 1.8 cm and 1971, 1.0 cm. The rainfall in both 1969 and 1970 appeared adequate, whereas the rainfall in 1971 provided less than adequate moisture for weed control. Preplant incorporated treatments of both atrazine and alachlor controlled yellow nutsedge (Table 1). In 1971 when the rainfall was less than adequate, the incorporation of the atrazine at 4.48 kg/ha provided greater yellow nutsedge control than the preemergence atrazine application. Pre-emergence application of alachlor at 4.48 kg/ha controlled more yellow nutsedge than did 2.24 kg/ha (Table 2). This was especially true for 1971 when rainfall was less than adequate. Both preplant and preemergence treatments of alachlor and atrazine combinations controlled 76 to 88% of the yellow nutsedge. A minimum rate of 3.36 kg/ha of alachlor was necessary for yellow nutsedge control. Preplant treatments with shallow incorporation or preemergence treatments of alachlor at 3.36 or 4.48 kg/ha controlled 80% or more of the yellow nutsedge. With less than adequate rainfall, preplant incorporated treatments of alachlor were more effective than preemergence treatments; but with adequate rainfall (1.27 to 2.54 cm rainfall within 10 days after

Table 1. Yellow nutsedge control 4 weeks after treatment from preplant incorporated herbicide treatments on a sandy loam soil with 2.8% organic matter.

Chemical	Rate (kg/ha)	Weed control rating ^a			
		(1969)	(1970)	(1971)	(mean)
Atrazine	4.48	9.0	9.7	9.7	9.6
Alachlor	3.36	8.3	8.7	9.0	8.7
Atrazine + alachlor	1.12 + 2.24	7.5	7.0	8.3	7.6
Atrazine + alachlor	1.12 + 4.48	8.2	8.7	8.3	8.4
Atrazine + alachlor	2.24 + 2.24	8.5	8.7	8.3	8.6

^a0-No control; 10-complete control or kill.

Table 2. Yellow nutsedge control 4 weeks after treatment from preemergence herbicide treatments on a sandy loam soil with 2.8% organic matter.

Chemical	Rate (kg/ha)	Weed control rating ^a			
		(1969)	(1970)	(1971)	(mean)
Atrazine	4.48	8.8	7.0	5.3	7.0
Alachlor	2.24	7.5	8.7	5.7	7.3
Alachlor	4.48	8.7	8.0	8.3	8.3
Atrazine + alachlor	2.24 + 2.24	8.5	8.0	7.7	8.1
Atrazine + alachlor	2.24 + 3.36	8.8	9.0	8.7	8.8
Atrazine + alachlor	1.12 + 2.24	8.5	8.3	7.0	7.9
Atrazine + alachlor	1.12 + 3.36	8.7	8.0	8.0	8.2

^a 0-No control; 10-complete control or kill.

herbicide application), both preemergence or preplant incorporated alachlor treatments effectively controlled yellow nutsedge. Postemergence alachlor applications on yellow nutsedge were not effective.

The depth of the tubers in the soil did not affect the response of yellow nutsedge to alachlor (Table 3). Yellow nutsedge shoot dry matter from tubers planted 2.5 cm which received preemergence alachlor applications was not significantly different from that of tubers planted 10.2 cm or 20.3 cm deep and received preemergence applications of alachlor. Both treatments significantly suppressed nutsedge growth. The three controls were significantly different in growth from each other because they emerged at three different times. Shoots emerged first from the tubers planted 2.5 cm deep then shoots emerged from the 10.2 cm deep tubers and finally the shoots emerged from tubers planted 20.3 cm deep.

The ryegrass bioassay for alachlor indicated that alachlor was present primarily in the top 5 cm of the loam soil indicating only limited leaching (Table 4).

The response of yellow nutsedge to alachlor was influenced by the percent organic matter present in the soil. The number of yellow nutsedge shoots emerging from soil with 5.55% organic matter or greater and treated with a preemergence application of 1.12 kg/ha of alachlor was

Table 3. Nutsedge response to alachlor 6 weeks after treatment as affected by depth of tubers in soil.

Tuber depth	Alachlor rate	Dry matter ^a
(cm)	(kg/ha)	(g/m ²)
2.5	0.00	154.6 a
	2.24	8.5 de
	4.48	10.6 de
10.2	0.00	123.7 b
	2.24	6.7 e
	4.48	3.2 e
20.3	0.00	68.5 c
	2.24	24.1 d
	4.48	5.9 e

^a Means with common letters are not significantly different at the 5% level, Duncan's Multiple Range Test.

Table 4. Ryegrass bioassay of alachlor leaching over a 6-week-period in greenhouse soil.

Depth of soil sample	Ryegrass growth in 2 wk	
	Alachlor (kg/ha)	
	2.24 ^a	4.48 ^a
(cm)	(mg fresh wt/plant)	
0.0 to 5.1	14.7 a	12.4 a
5.1 to 10.2	28.0 b	24.4 b
10.2 to 15.2	26.1 b	21.7 b

^a Means within columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

similar to the number of shoots emerging in soils not receiving alachlor (Table 5). The 2.84% and 4.57% organic matter soils had significantly less shoots emerging when alachlor was applied. Preemergence application of alachlor at 1.12 kg/ha regardless of percent organic matter did not reduce shoot height. Alachlor applied at 3.36 kg/ha to soils with 5.55% or less organic matter significantly reduced shoot emergence. Four weeks after either 1.12 kg/ha or 3.36 kg/ha alachlor application the shoot height for yellow nutsedge plants were similar, except in the high organic matter soils (Figure 1). But after 8 weeks of growth, only the yellow nutsedge grown in low organic matter soils with less than 5.55% organic matter had significantly reduced shoot height (Table 5 and Figure 2).

Alachlor has no significant effect on tuber sprouting, but it significantly reduced shoot elongation (Table 6). Concentrations of 3.7×10^{-6} M or 18.5×10^{-5} M alachlor did not affect the sprouting of the yellow nutsedge tubers. The low concentration of 3.7×10^{-6} M alachlor dramatically prevented the growth of yellow nutsedge shoots (Figure 3). After only 8 hr of treatment, shoot length was significantly reduced (Table 7).

Following the treatment of tubers with 3.7×10^{-6} M alachlor for 1 week, more shoots emerged after 4 weeks

Table 5. Response of yellow nutsedge to preemergence alachlor and various levels of organic matter 8 weeks following treatments.

Alachlor (kg/ha)	Organic matter (%)	Emergence ^a (shoots/95 cm ²)	Shoot height ^a (cm)
0.00	2.84	14.9 fg	24.0 c
	4.57	13.1 fg	25.9 cd
	5.55	12.4 fg	24.1 c
	10.28	13.4 fg	23.3 bc
	81.40	18.1 h	25.8 c
1.12	2.84	7.6 cd	19.2 b
	4.57	9.2 de	20.5 bc
	5.55	12.4 fg	24.9 c
	10.28	13.8 fg	22.6 bc
	81.40	19.1 h	24.9 c
3.36	2.84	1.9 a	5.6 a
	4.57	3.6 ab	10.8 a
	5.55	5.8 bc	17.5 b
	10.28	11.3 eg	21.2 bc
	81.40	9.3 de	20.2 bc

^a Means within columns with common letters are not significantly different at the 5% level, Duncan's Multiple Range Test.

Figure 1. Response of yellow nutsedge to alachlor and various levels of organic matter 4 weeks following treatment.

0.00 KG/HA**2.8 4.6 5.6 10.3 81.4 %0.M.****1.12 KG/HA****2.8 4.6 5.6 10.3 81.4 %0.M.****3.36 KG/HA****2.8 4.6 5.6 10.3 81.4 %0.M.**

Figure 2. Response of yellow nutsedge to alachlor and various levels of organic matter 8 weeks following treatment.

0.00 KG/HA



2.8 4.6 5.6 10.3 81.4 %0.M.

1.12 KG/HA



2.8 4.6 5.6 10.3 81.4 %0.M.

3.36 KG/HA



2.8 4.6 5.6 10.3 81.4 %0.M.

than when alachlor was present for the entire 4 weeks (Table 8). Apparently alachlor upset apical dominance in tubers. Shoot length was significantly different among all three treatments: no alachlor present, alachlor present for only one week, and alachlor present for four weeks (Figure 4). The tallest shoots occurred when no alachlor was present, and minimum shoot growth resulted from 4 weeks of alachlor treatment. Alachlor did not kill the tubers, it suppressed shoot growth from these tubers.

Alachlor is an effective herbicide for controlling yellow nutsedge growth if it is present in the soil during the time of tuber sprouting. Yellow nutsedge control with alachlor is dependent on proper timing and placement of the herbicide and adequate rainfall after application.

Table 6. Effect of alachlor on nutsedge tuber sprouting and shoot growth 5 days after treatment.

Alachlor	Sprouting ^a	Shoot elongation ^a
(conc)	(%)	(mm/plant)
0	94.5 a	15.4 a
3.7×10^{-6} M	94.3 a	3.3 b
18.5×10^{-5} M	93.3 a	2.6 b

^a Means within columns with common letters are not significantly different at the 5% level, Duncan's Multiple Range Test.

Figure 3. Effect of alachlor on nutsedge shoot growth 5 days after treatment.

Alachlor (Moles/Liter)

0

3.7 x 10⁻⁶



Table 7. Effect of tuber exposure time to 3.7×10^{-6} M alachlor on nutsedge shoot length.

Exposure time to alachlor	Nutsedge shoot length ^a			
	1 wk (mm/plant)	2 wk (mm/plant)	3 wk (mm/plant)	4 wk (mm/plant)
(hr)				
0	14.5 c	57.8 c	85.5 d	87.5 d
8	9.5 b	25.8 b	38.5 c	37.8 bc
24	4.2 a	11.5 ab	15.7 abc	16.3 abc
48	2.5 a	15.0 ab	33.8 bc	42.2 c
72	2.8 a	6.8 a	11.0 ab	12.0 ab
96	2.3 a	7.3 a	14.2 abc	19.2 abc
120	1.3 a	4.3 a	16.3 abc	19.8 abc
144	3.2 a	4.5 a	5.0 a	5.3 a

^a Means within columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 8. New shoot emergence and growth of yellow nutsedge from sand cultures containing sprouted tubers exposed to 3.7×10^{-6} M alachlor.

Treatment	New shoot emergence ^a	Shoot length ^a
	(number/culture)	(cm/shoot)
No treatment	0.9 ab	19.1 a
Alachlor for 1 week	2.1 a	10.0 b
Alachlor for 4 weeks	0.0 b	0.9 c

^a Means within columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Figure 4. New shoot emergence and growth of yellow nutsedge from sand cultures containing sprouted tubers exposed to 3.7×10^{-6} M alachlor, C = no treatment, A = alachlor for 1 week, and B = alachlor for 4 weeks.

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CHAPTER 3

The Absorption, Translocation, and Metabolism of Alachlor by Yellow Nutsedge

Abstract

The main site of uptake of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide (alachlor) by small yellow nutsedge (Cyperus esculentus L.) plants is the tissue above the tuber. Primarily acropetal and some basipetal ^{14}C translocation occurred from ^{14}C -alachlor applications to small seedlings with 2 to 4 cm shoots. Alachlor is metabolized rapidly in yellow nutsedge to at least one water-soluble metabolite. Applications of ^{14}C -alachlor to 15 cm shoots resulted in limited acropetal ^{14}C translocation.

Introduction

The perennial, yellow nutsedge, is a serious weed problem in the United States and many other parts of the world (2, 3, 9, 10).

Yellow nutsedge has numerous slender rhizomes ending in tubers or shoots (1). Tubers in the soil will produce tuber rhizomes which may form basal bulbs below the soil surface (5, 8). Shoots, rhizomes, and roots

are produced from a basal bulb (5, 8). Thus, there are many potential sites for alachlor uptake by yellow nutsedge to explain growth inhibition (4, 12). Alachlor also delays nutsedge emergence and reduces tuber production.^{1,2}

Alachlor uptake has been reported to be greater in the susceptible species, barley, than tolerant corn.³ Alachlor was absorbed by the roots and then translocated in the xylem to the tips of the older leaves. In barley, more alachlor was present in the shoot than in the root following treatment. Thin layer chromatography (TLC) of the water-soluble extracts from barley shoots and roots showed that most of the ^{14}C was found in one major metabolite and one minor metabolite occurring in each shoot and root.

Similarly, 2-chloro-N-isopropylacetanilide (propachlor) was metabolized to water-soluble metabolites in corn and barley (7). Both resistant and susceptible species have the ability to metabolize chloroacetamides (6). The basis for selectivity in the case of chloroacetamide herbicides may be the ability

¹Godke, D. L. 1969. The control of yellow nutsedge with alachlor and an alachlor plus linuron mixture. M. S. Thesis. Southern Illinois University, Carbondale. 58p.

²Worthington, J. P. 1971. The effect of alachlor and MON-097 on the growth of yellow nutsedge and the uptake of ^{14}C -labeled alachlor by yellow nutsedge and soybeans. Ph.D. Thesis. Ohio State University, Columbus. 62p.

³Hamill, A. S. 1971. Bases for the interaction of alachlor, butylate or chlorbromuron with carbofuran on barley and corn. Ph.D. Thesis. Michigan State University, E. Lansing. 126p.

of resistant plants to metabolize them at a rate sufficient to keep their levels below that required for growth inhibition (6).

The objective of this study was to determine the site of uptake, the translocation, and the metabolism of alachlor in order to explain growth inhibition in young yellow nutsedge plants.

Materials and Methods

The site of alachlor uptake was determined by applying alachlor separately to yellow nutsedge roots or shoots. An activated charcoal layer was used to isolate the root and shoot regions to prevent herbicidal movement (Figure 1 and 2). Alachlor was applied at 0, 1.12 or 3.36 kg/ha above or below the charcoal barrier. Water was surface applied throughout the duration of the experiment to maintain soil moisture for nutsedge growth. Shoot emergence and height were recorded at both 2 and 4 weeks after the start of the treatment. The values reported in Table 1 are the means of two experiments with ten replications per experiment.

Uniformly ring-labeled ^{14}C -alachlor was applied to a 15 cm tall yellow nutsedge plant to study herbicidal translocation. The ^{14}C -alachlor had a specific activity of 1.02 μC per μmole . Each plant received a 5 μl drop containing 0.25 μC placed on a mature leaf. After 2 days of treatment, the plants were freeze dried and radioautographed.

The translocation of ^{14}C -alachlor was studied in small yellow nutsedge seedlings with shoots 2 to 4 cm in length above soil surface by applying 5 μl with 0.25 μC of ^{14}C -alachlor to one of three different sites of application; on the shoot above the growing point, on the tuber rhizome below the growing point or on the roots (Figure 3).

Figure 1. Charcoal barrier method that separates alachlor treated soil from untreated soil.

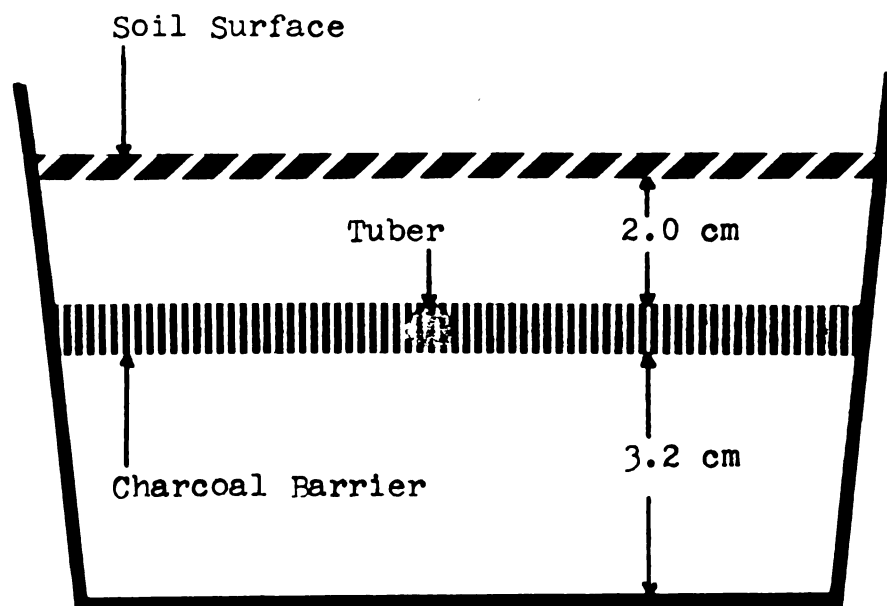
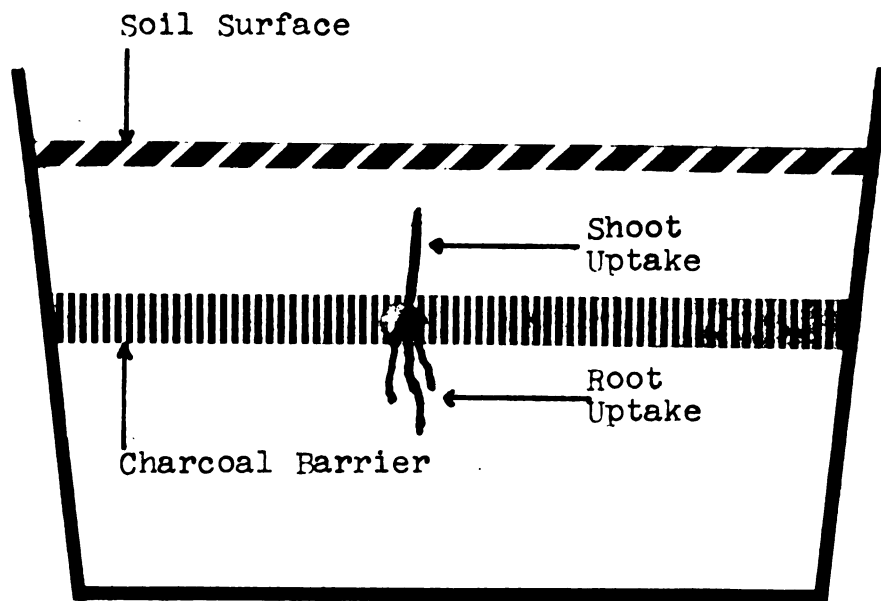


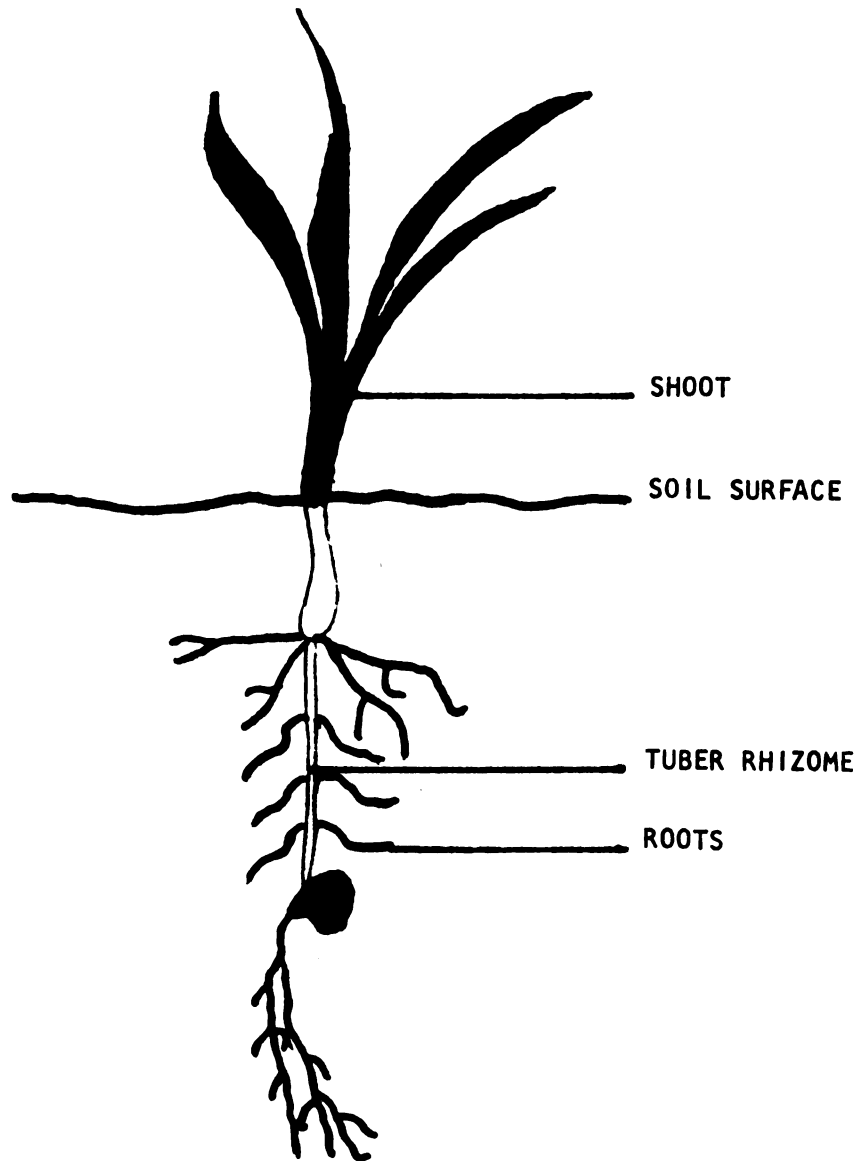
Figure 2. Diagram of charcoal barrier method showing shoot or root alachlor uptake.



After 2 days of treatment the experiment was terminated and the plants radioautographed. The plants were then homogenized and extracted for ^{14}C -alachlor and metabolites by method of Hamill.⁴ The samples were extracted for ^{14}C -labeled materials for 6 hr with 10 ml of 80 percent acetone. To facilitate extraction the vials containing the samples were placed in a reciprocating shaker to agitate the sample. Following extraction the vials were centrifuged at 455 x g for 5 min. The supernatant fluid was removed, stored, and the pellet extracted as before for 8 hr, centrifuged, and the corresponding supernatants fluids combined. The supernatant volume was reduced under nitrogen at 30 C. After a third extraction with 10 ml of 100 percent acetone for 10 hr, the homogenate was filtered through No. 1 Whatman filter paper. The filtrate was added to the previously obtained supernatant fluid. The volume of the combined filtrate-supernatant was reduced under nitrogen to 4 ml. One ml of hexane was added to the extract with a Vortex test tube stirrer. The mixture was centrifuged at 455 x g for 10 min, the vials sealed, and then placed in a freezer for 1 hr to facilitate separation of the hexane and aqueous fractions. Both the hexane and aqueous fractions were assayed for radioactivity with a scintillation spectrometer.

⁴Ibid.

Figure 3. Sites of application of ^{14}C -alachlor to yellow nutsedge plants.



A portion of the dried acetone-insoluble residue was weighed and combusted for radioassay by the Schoeniger combustion method of Wang and Willis (13).

Metabolism was studied by applying ^{14}C -alachlor to 4 to 6 cm shoots as previously described. After 5 days of treatment, the plants were homogenized in 20 ml of 80 percent acetone with a Waring blender for 2 min and filtered through No. 1 Whatman filter paper. The filtrate was reduced to a 2.0 ml aqueous fraction. Two ml of hexane was added to the filtrate, mixed, and then put in a freezer over night to facilitate separation of the two fractions. The volume of each fraction was reduced to 0.1 ml and 50 μl of each were spotted on 250 micron thick silica gel H thin layer plates. The plates were developed in either a polar or a non polar solvent system. The nonpolar solvent system was petroleum ether:chloroform:95 percent ethanol (7:2:1; v/v/v); and the polar solvent system was 1-butanol:glacial acetic acid:water (12:3:5; v/v/v). After development for 15 cm on the plate, the plates were divided into 1 cm bands and the radioactivity determined as previously described.

Results and Discussion

Reduction in yellow nutsedge shoot growth occurred when alachlor was absorbed from the soil by the tissue above the tuber (Table 1). Both emergence of shoots and shoot height were affected. The absorption of alachlor by roots did not reduce plant growth (Figure 4).

Foliar application of ^{14}C -alachlor resulted in limited acropetal and no basipetal translocation of alachlor in 15 cm tall plants (Figure 5). Postemergence applications of alachlor probably do not control yellow nutsedge because alachlor is not translocated to the growing point.

When ^{14}C -alachlor was applied on the shoot, tuber rhizome, or root of small nutsedge seedlings (Figure 6) differences in translocation occurred (Figure 7). The ^{14}C -material translocated from the shoot and tuber rhizome to the growing point, but the majority of the ^{14}C from ^{14}C -alachlor applied to the root did not move. Alachlor was absorbed by the shoots of very young plants, and was translocated acropetally or basipetally to the yellow nutsedge growing points killing the plants.

Two days after ^{14}C -alachlor application, 81 percent of the ^{14}C -labeled material was metabolized by yellow nutsedge to a water-soluble product (Tables 2 and 3).

Only one major water-soluble metabolite of alachlor was present in the water-soluble fraction after 5 days.

This is similar to Hamill's results showing rapid alachlor metabolism to a water-soluble product in barley, a susceptible species.

Alachlor absorption by small nutsedge seedlings through the shoot or tuber rhizome and subsequent translocation to the growing point appears responsible for reduced emergence of shoots, shoot height, and eventual death of the young plant.

Table 1. Site of alachlor uptake by yellow nutsedge.

Alachlor rate (kg/ha)	Location of herbicide	Shoot emergence ^a		Shoot height ^a	
		2 wk	4 wk	2 wk	4 wk
		(%)	(%)	(cm/plant)	
0	-	75 c	95 c	3.85 b	10.4 b
1.12	above barrier	30 b	50 b	0.22 a	1.1 a
3.36	above barrier	0 a	15 a	0.00 a	0.0 a
1.12	below barrier	95 d	100 c	5.01 c	9.8 b
3.36	below barrier	95 d	95 c	5.32 c	11.4 b

^a Means within columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Figure 4. Site of alachlor uptake by yellow nutsedge
C = control, LA1 and LA3 = alachlor applied above the
tuber at 1.12 and 3.36 kg/ha respectively, LB1 and
LB3 = alachlor applied below the tuber at 1.12 and
3.36 kg/ha respectively.

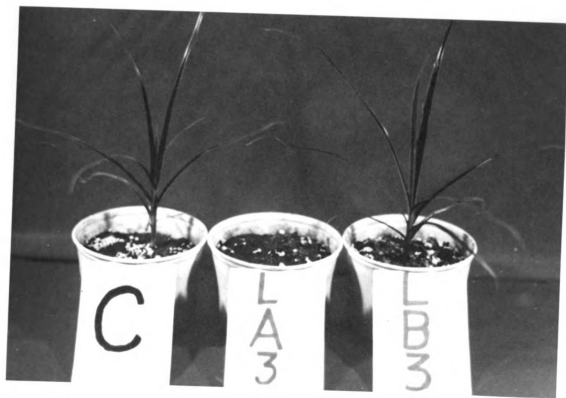
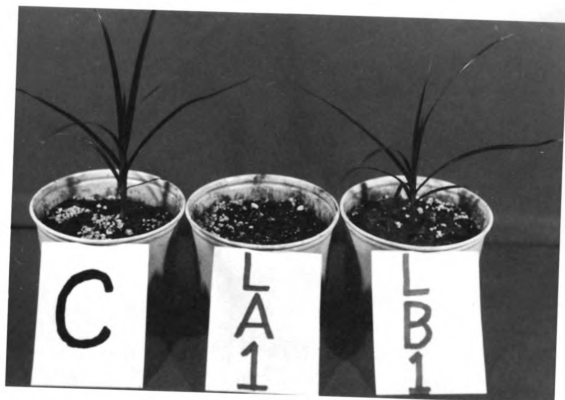


Figure 5. Translocation of ^{14}C -alachlor in 15 cm yellow nutsedge plants. Left: nutsedge plant treated with ^{14}C -alachlor. Right: radioautograph of ^{14}C -alachlor treated plant. Rectangular area is where ^{14}C -alachlor was applied.

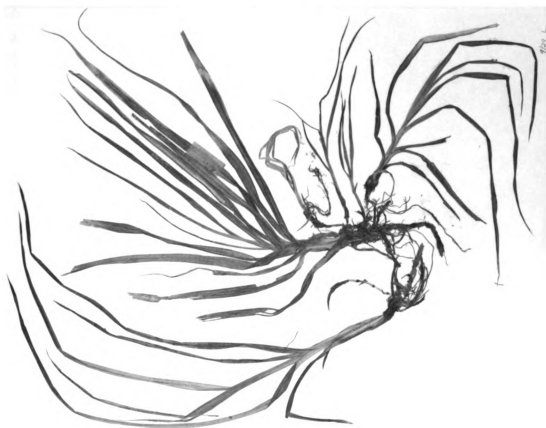
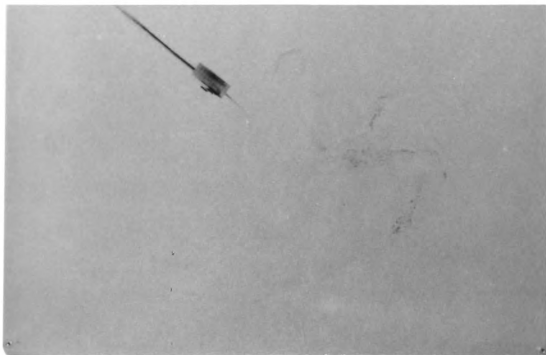
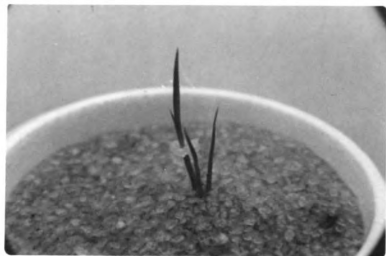
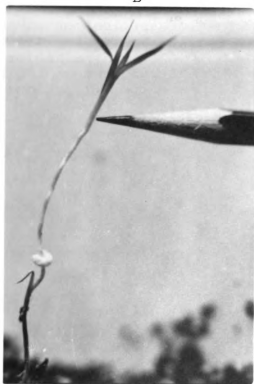


Figure 6. Sites of ^{14}C -alachlor application on yellow nutsedge seedlings. A = alachlor applied on small shoot above growing point. B = alachlor applied on tuber rhizome. Pencil indicates location of growing point. C = root application of alachlor.

A



B

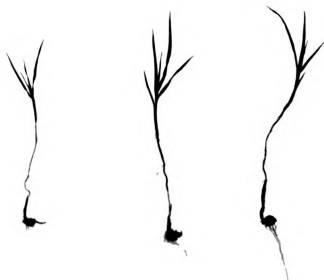


C



•

Figure 7. Translocation of ^{14}C -alachlor from three sites of application. Top = nutsedge treated plants and Bottom = radioautographs of treated plants. A, B and C = shoot, tuber rhizome and root application of ^{14}C -alachlor.



A

B

C

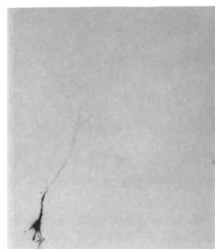


Table 2. The partitioning and distribution of ^{14}C in 10-day-old yellow nutsedge 2 days after ^{14}C -alachlor application.

Treatment	Acetone-insoluble ^a	Water-soluble ^a	Hexane-soluble ^a
	(dpm/mg)	(dpm/mg)	(dpm/mg)
Shoot			
Above growing point	1426 b	25461 b	6321 b
Growing point	790 a	2040 a	390 a
Below growing point	13 a	117 a	26 a
Tuber rhizome			
Above ^{14}C spot	337 b	2796 a	164 b
Below ^{14}C spot	11 a	17 a	20 a
Root			
Above ^{14}C spot	94 b	451 a	70 b
Below ^{14}C spot	15 a	53 a	22 a

^a Means within treatment columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Table 3. The separation by TLC of ^{14}C -extracts from shoots of 11-day-old yellow nutsedge 5 days after ^{14}C -alachlor treatment.^a

Treatment	Percent of total spotted												
	Rf	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	
Water-soluble fraction	81									3 ^b			
Hexane-soluble fraction	2									14 ^b			

^a TLC plates were developed in petroleum ether:chloroform:95 percent ethanol (7:2:1; v/v/v).

^b The ^{14}C -alachlor standard co-chromatographed at this Rf.

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CHAPTER 4

SUMMARY AND CONCLUSIONS

Field, greenhouse and laboratory studies were conducted to evaluate the response of yellow nutsedge to alachlor, and determine the site of uptake, the translocation and the metabolism of alachlor by yellow nutsedge.

The results of these investigations are summarized as follows:

1. Preplant incorporated or preemergence applications of alachlor at 3.36 or 4.48 kg/ha effectively controlled yellow nutsedge.
2. With less than adequate rainfall (less than 2.5 cm with in 7 to 10 days after treatment) preplant incorporated alachlor treatments were more effective than preemergence applications.
3. Alachlor did not effectively control yellow nutsedge in high organic matter soils.
4. Reduction in yellow nutsedge shoot growth occurred when alachlor was absorbed from the soil by the tissue above the tuber.
5. Absorption of alachlor by roots did not reduce plant growth.
6. Alachlor at 3.7×10^{-6} M inhibited growth of yellow nutsedge shoots in Petri dish cultures.

7. Alachlor at 3.7×10^{-6} M killed newly emerging nutsedge shoots in Petri dish cultures.
8. Alachlor at 3.7×10^{-6} M or 18.5×10^{-5} M did not inhibit sprouting of yellow nutsedge tubers.
9. Depth of tubers in the soil did not affect nutsedge response to alachlor.
10. Six weeks after treatment, alachlor that was still present in a loam soil was primarily in the top 5 cm of soil.
11. Primarily acropetal and some basipetal ^{14}C -translocation occurred from ^{14}C -alachlor applications to small yellow nutsedge seedlings with 2 to 4 cm shoots.
12. Alachlor was absorbed by 10-day-old plants and translocated acropetally or basipetally to the yellow nutsedge growing points killing the plants.
13. After ^{14}C -alachlor treatments on small nutsedge seedlings, the ^{14}C -material moved from the shoot and tuber rhizomes to the growing point, but the majority of the ^{14}C from ^{14}C -alachlor applied to the root did not move.
14. In tall (15 cm shoots) yellow nutsedge plants, limited acropetal and no basipetal ^{14}C -translocation occurred.

15. Postemergence alachlor applications do not control yellow nutsedge, because alachlor is not translocated to the growing point.
16. Alachlor is metabolized rapidly in yellow nutsedge to at least one water-soluble metabolite.
17. Alachlor is an effective herbicide for controlling yellow nutsedge in Michigan.

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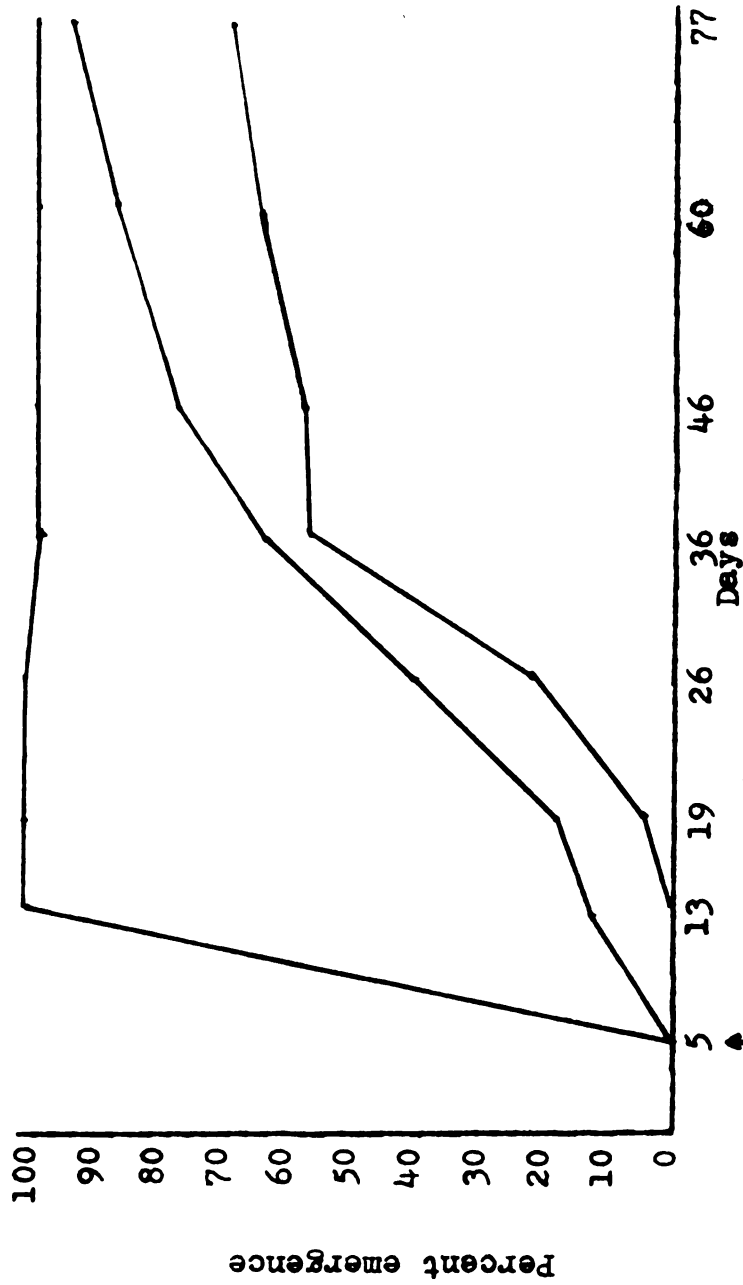
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APPENDICES

Appendix A



Transplanted sprouted tubers

Shoot emergence from pots containing sprouted tubers which received a 5-day treatment of one of the following: (A) control, no alachlor, (B) 3.7×10^{-6} M alachlor, and (C) 18.5×10^{-5} M alachlor.

Appendix B

Response of yellow nutsedge tuber sprouting
to 3.7×10^{-6} M alachlor treatments.

Exposure time to alachlor	Percent sprouting ^a			
(hr)	(1 wk)	(2 wk)	(3 wk)	(4 wk)
0	30.0 c	43.3 a	44.2 a	44.2 a
8	30.0 c	58.3 a	68.2 a	68.2 a
24	23.3 bc	38.7 a	38.7 a	38.7 a
48	16.7 abc	29.2 a	30.0 a	30.0 a
72	23.3 bc	43.3 a	47.0 a	47.0 a
96	8.3 ab	30.5 a	33.3 a	37.5 a
120	6.7 a	22.0 a	31.2 a	36.2 a
144	30.8 c	47.0 a	48.7 a	48.7 a

^a Means within columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Appendix C

New shoot emergence and growth of yellow nutsedge
from sand cultures containing sprouted tubers
exposed to 3.7×10^{-6} M alachlor.

Treatment	New shoot emergence ^a		Shoot length ^a	
	3 wk (No./culture)	4 wk (No./culture)	3 wk (cm/shoot)	4 wk (cm/shoot)
No treatment	0.0 b	0.9 ab	11.9 b	19.1 a
Alachlor for 1 week	0.8 ab	2.1 a	2.0 c	10.0 b
Alachlor continously	0.0 b	0.0 b	0.3 c	0.9 c

^a Means within columns with common letters are not significantly different at the 5% level by Duncan's Multiple Range Test.

Appendix D

The separation by TLC of extracts from shoots of 11-day-old yellow nutsedge 5 days after ^{14}C -alachlor treatment.^a

Treatment	Rf										
	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Water-soluble fraction	0.0										
Hexane-soluble fraction							0.68				
^{14}C -alachlor ^b							0.68				

^a TLC plates were developed in petroleum ether:chloroform:95 percent ethanol (7:2:1; v/v/v).

^b Ring labeled ^{14}C -alachlor was used as the standard.

Appendix E

The separation by TLC of water soluble alachlor metabolites of 11-day-old yellow nutsedge 5 days after ^{14}C -alachlor treatment.^a

	RF											
Treatment	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	

Water-soluble metabolites	0.33 ^b											
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^a TLC plates were developed in 1-butanol:glacial acetic acid: water (12:3:5).

^b Ring labeled ^{14}C -alachlor was used as the standard and had an Rf of 0.60.

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