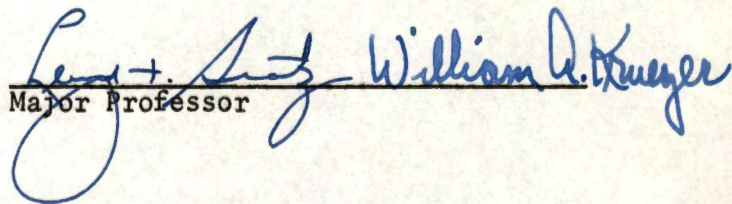


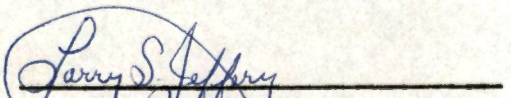
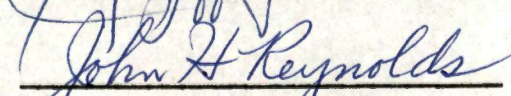
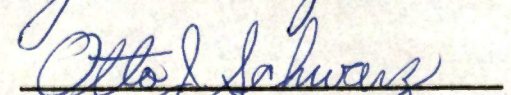
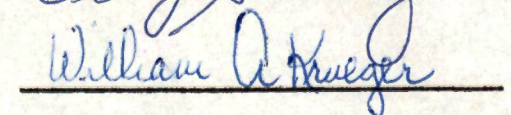
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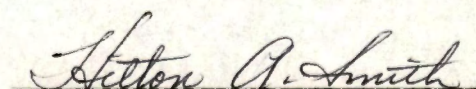
I am submitting herewith a dissertation written by Jesse Stephen Hickey entitled "Effect of Alachlor and 1,8-Naphthalic Anhydride on the Growth and Development of Grain Sorghum and Corn Seedlings." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Science.


Major Professor

We have read this dissertation
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CRANESEST CREST

EFFECT OF ALACHLOR AND 1,8-NAPHTHALIC ANHYDRIDE ON THE GROWTH
AND DEVELOPMENT OF GRAIN SORGHUM AND CORN SEEDLINGS

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Jesse Stephen Hickey

December 1973

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ABSTRACT

Morphological, physiological, and biochemical parameters were measured to determine the site of action of alachlor [2-chloro-2',6' diethyl-N-(methoxymethyl) acetanilide] and the protective effect of 1,8-naphthalic anhydride (hereafter referred to as NA) in grain sorghum (Sorghum bicolor (L.) Moench, 'AKS 614') and corn (Zea mays L. 'Tn 5009') seedlings. Time of coleorhiza emergence through the pericarp of grain sorghum was not affected by 1, 5, or 25 ppmw alachlor, by 2 percent w/w NA, or their combinations. Coleoptile emergence was delayed slightly by NA alone and in combination with alachlor, but not by alachlor alone. Emergence of the primary leaf from the coleoptile was inhibited by alachlor treatments in the presence of NA and even more so in its absence. Various degrees of leaf distortion resulted when grain sorghum seedlings not exhibiting leaf emergence were placed in light. Severity of distortion was less with 1 ppmw alachlor than with 5 ppmw and the alachlor-induced distortion was less when in combination with 150 ppmw NA. After four weeks, the primary leaves were still distorted but subsequent emerging leaves were normal in all treatments. More complete emergence and unrolling of the primary leaf was observed when terminal segments (3.5 cm long, including the coleoptile and part of the first internode) of grain sorghum grown in the presence of 150 ppmw NA were vacuum infiltrated with 15, 25, and 50 ppmw alachlor than in the controls. Fifty ppmw gibberellic acid (GA) was as effective as 150 ppmw NA in counteracting alachlor-induced leaf retention in the

coleoptiles of corn. The GA was also effective in nullifying the height reduction observed in corn coleoptiles grown in the presence of 25 ppmw alachlor and 150 ppmw NA, while 150 ppmw NA applied in combination with the alachlor further reduced coleoptile length. One-cm coleoptile segments from corn grown in the presence of 25 ppmw alachlor, 150 ppmw NA and their combination responded more to indoleacetic acid (IAA)-induced elongation than did the controls. More force was required to induce leaf slippage in coleoptiles from corn grown in the presence of 25 ppmw alachlor than in the controls, or in coleoptiles from plants grown in the presence of 25 ppmw alachlor in combination with 150 ppmw NA. The NA alone reduced the force required to induce leaf slippage below that of all treatments compared. Incubation for 16 hours in 10 ppmw IAA did not lower the amount of force required to induce leaf slippage in coleoptiles from corn grown in the presence of 25 ppmw alachlor even though the length of the coleoptiles increased 30 percent more than coleoptiles not receiving IAA treatment. Percent dry matter, dry matter per 100 plants, and percent protein increased in coleoptiles from alachlor treated corn, while percent cellulose and lignin decreased. The extent of variation in these parameters was reduced when NA was applied in combination with alachlor, with the exception of percent lignin which was further decreased by the combination treatment. Percent hemicellulose was not affected by alachlor, NA, or their combinations. The RNA-DNA ratio of corn coleoptiles was decreased by the alachlor-NA combination treatment, but not by either chemical alone.

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CHAPTER I

INTRODUCTION

A recent development which has created much excitement among agricultural chemical users is that of using one chemical to protect a plant from another chemical used in producing the crop. One of the earliest antidotal compounds which appeared to be economically feasible to use was reported by Gulf Chemical Company researchers who reported that NA seed treatment could reduce plant damage in grain sorghum by alachlor and in corn by S-ethyl dipropylthiocarbamate (EPTC). A compound affording such protection is desirable in a modern weed control program for these crops as currently recommended herbicides have certain disadvantages. Among these disadvantages are specific moisture requirements for activation, soil type restrictions, and the limited control spectra of these herbicides (e.g., johnsongrass is not adequately controlled in corn by atrazine). As is true for most of the chemicals used for plant growth regulation, the mode of action by which NA protects grain sorghum and corn from herbicide injury is not known. The objective of this research was to obtain information regarding the site of action of the protective effect of NA and to study the effects of alachlor in the presence and absence of NA on certain morphological, physiological, and biochemical processes in grain sorghum and corn seedlings.

CHAPTER II

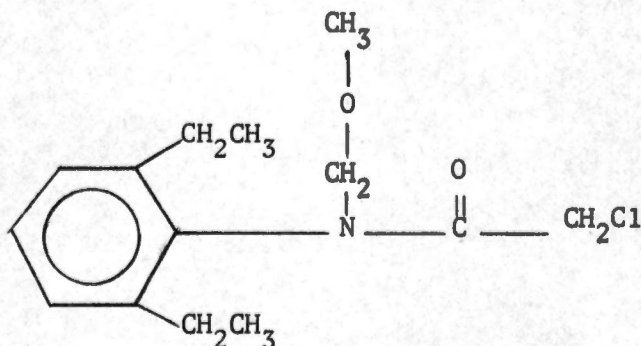
LITERATURE REVIEW

This review will consider three aspects of the alachlor-NA interaction:

1. Alachlor; its chemistry, herbicidal properties, control spectrum, physiological and biochemical effects and postulated mechanisms of action.
2. Herbicidal plant protectants; a review of earlier compounds exhibiting antidotal properties, NA's antidotal properties and its protective spectrum.
3. The alachlor-NA interaction; effects on crop injury and yield and possible modes of action.

I. ALACHLOR

Chemistry



2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide

Alachlor has a water solubility of 140 ppmw at 23 C. The acute oral LD₅₀ to male and female rats is 1,200 mg/kg and the minimum lethal dose by skin absorption is greater than 2,000 mg/kg (82). Residual life of the chemical in the soil is relatively short with a half life of 14-16 days (28).

Herbicidal Properties

Alachlor was introduced by the Monsanto Company at a soybean seminar in St. Louis, Missouri on January 16, 1969 as a new selective herbicide for soybeans. The compound was reported to be effective under a wide range of soil and moisture conditions. It does not require soil incorporation, therefore loss of moisture is minimized, and the cost of application is minimal. The short residual life allows growers to adopt any crop rotation program, without risk of herbicide carryover (74).

Resistant plants. Alachlor is used in several agronomic crops including corn, peanuts, and soybeans (82). Cotton (Gossypium hirsutum L.) has also been shown to be moderately tolerant to preemergence treatments of alachlor (6,18,24,26,28,50,71). Various plants exhibiting some tolerance to preemergence treatments of alachlor are listed in Table 1. Most plants which are tolerant to preemergence applications of alachlor are also tolerant to postemergence alachlor treatments (6,28,32,59).

Control spectrum. Alachlor is highly active on many annual grasses and several broadleaf weeds. Weeds susceptible to this herbicide are listed in Table 2. Alachlor's control of annual morning glory

TABLE 1
PLANTS EXHIBITING TOLERANCE TO ALACHLOR

Common Name	Scientific Name	Reference
<u>Crops</u>		
Beans		
dwarf	<u>Phaseolus</u> spp.	6,68
lima	<u>P. limensis</u> Macf.	6,33
snap	<u>P. vulgaris</u>	6,71
Corn	<u>Zea mays</u> L.	3,6,21,23,26, 27,28,46,74
Cabbage	<u>Brassica oleracea</u> L.	6,27,28,71
Peanuts	<u>Arachis hypogaeae</u> L.	5,12,21,23,26, 27,28,71,74
Peas	<u>Pisum</u> spp.	3,6,26
Potatoes	<u>Solanum tuberosum</u> L.	6,31,58
Radishes	<u>Raphanus sativus</u> L.	6,27,28,71
Rape	<u>Brassica</u> spp.	6,18,21,24,26, 27,28,45,71
Soybeans	<u>Glycine max</u> Merrill	6,27
Sugar Cane	<u>Saccharum officinarum</u> L.	13,28,77
Tomatoes	<u>Lycopersicon esculentum</u> L.	6
<u>Weeds</u>		
Prostrate knotweed	<u>Polygonum aviculare</u> L.	28
Wild buckwheat	<u>P. convolvulus</u> L.	28
Ladysthumb	<u>P. persicaria</u> L.	28
Hoary cress	<u>Cardaria draba</u> (L.) Desv.	28
Hairy whitetop	<u>C. pubescens</u> (C.A.Mey.) Rollins	28
Wild radish	<u>Raphanus raphanistrum</u> L.	28

TABLE 2
WEEDS SUSCEPTIBLE TO ALACHLOR

Common Name	Scientific Name	Reference
Annual bluegrass	<u>Poa annua</u> L.	28
Barnyardgrass	<u>Echinochloa crus-galli</u> (L.) Beauv.	6,11,18,21,23, 27,28,46
Black nightshade	<u>Solanum nigrum</u> L.	23,28
Brachiaria	<u>Brachiaria</u> spp. L.	23
Canada thistle	<u>Cirsium arvense</u> (L.) Scop.	28
Carpetweed	<u>Mollugo verticillata</u> L.	23,74
Catchweed bedstraw	<u>Galium aparine</u> L.	28
Chickweed	<u>Stellaria media</u> (L.) Cyrille.	28
Common groundsel	<u>Senecio vulgaris</u> L.	28
Common lambsquarter	<u>Chenopodium album</u> L.	6,11,23,28
Common purslane	<u>Portulaca oleracea</u> L.	6,23,28
Corn speedwell	<u>Veronica arvensis</u> L.	28
Corn spurry	<u>Spergula arvensis</u> L.	28
Crabgrass	<u>Digitaria</u> spp.	5,6,12,21,23, 27,28,46,74
Downy brome	<u>Bromus tectorum</u> L.	46
Field pennycress	<u>Thlaspi arvense</u> L.	28
Florida beggarweed	<u>Desmodium tortuosum</u> (Sw.) DC.	23
Florida pusley	<u>Richardia scabra</u> L.	5,21,23,74
Foxtail	<u>Setaria</u> spp.	6,11,23,30,46,51
Fumitory	<u>Fumaria officinalis</u> L.	28
Goosegrass	<u>Eleusine indica</u> (L.) Gaertn.	21,23
Hempnettle	<u>Galeopsis tetrahit</u> L.	28
Horsetail	<u>Equisetum</u> spp. L.	28
Johnsongrass (seedling)	<u>Sorghum halepense</u> (L.) Pers.	23
Mayweed	<u>Anthemis cotula</u> L.	28
Panicum	<u>Panicum</u> spp. L.	6,23,30,44
Prickly sida	<u>Sida spinosa</u> L.	17,23,28
Ragweed	<u>Ambrosia</u> spp. L.	6,28
Red deadnettle	<u>Lamium purpureum</u> L.	23,28
Red rice	<u>Oryza sativa</u> L.	6
Redroot pigweed	<u>Amaranthus retroflexus</u> L.	5,11,18,21,23,24, 28,30,46,62,74
Red sorrel	<u>Rumex acetosella</u> L.	28
Scarlet pimpernel	<u>Anagallis arvensis</u> L.	28
Shepherdspurse	<u>Capsella bursa-pastoris</u> (L.) Medic	6,28

TABLE 2 (continued)

Common Name	Scientific Name	Reference
Smallflower galinsoga	<u>Galinsoga parviflora</u> Cav.	28
Spiny sowthistle	<u>Sonchus asper</u> (L.) Hill	28
Spreading orach	<u>Atriplex patula</u> L.	28
Wild oat	<u>Avena fatua</u> L.	46
Yellow nutsedge	<u>Cyperus esculentus</u> L.	9,23

(Ipomoea spp. L.) (7,12,17,45,76) and cocklebur (Xanthium pensylvanicum Wallr.) is erratic (7,76).

The control spectrum of alachlor may vary slightly with method of application. Benson et al. (11) reported that alachlor is as effective preplant incorporated as surface applied when used at higher rates both for the control of annual grasses and certain broadleaf weed species. Soil incorporation increased alachlor's control of yellow nutsedge.

Stickler et al. (75) however, reported that increasing soil moisture did not significantly increase the effectiveness of alachlor's control of giant foxtail. They concluded that benefits from soil incorporation of alachlor would be questionable. Spurrier and Carpenter (74) found that preplant incorporation was a satisfactory method of applying alachlor in the southwest, however, in Alabama and Georgia, soil incorporation diluted its activity. They also observed that alachlor applied to emerged weeds or grasses in peanuts at cracking time did not provide adequate control.

Hargrove and Merkle (37) noted that alachlor provided poor weed control if hot, dry weather occurs following its application. In a study initiated to determine the influence of soil temperature and relative humidity on the degradation and volatilization of alachlor, they found that alachlor was degraded to 2-chloro-2'6'-diethylacetanilide under conditions of low humidity and high temperatures. They concluded that alachlor's reduced effectiveness when application is followed by hot dry conditions was due to this chemical degradation. The loss of alachlor at higher relative humidities was attributed to volatilization, not degradation.

Effective Site of Uptake

The effective site of uptake of alachlor appears to differ in monocots and dicots. Knake and Wax (51), using giant foxtail in a split-pot bioassay, reported that alachlor was just as effective in the shoot zone as in the seed zone. Results of Eshel (26), on the other hand, indicated that the main entrance of alachlor to cotton plants is through the roots. Eshel suggests that in the monocots such as the giant foxtail used by Knake and Wax, the first internode, adventitious roots, and coronal roots developing from the coleoptile node were included in the above seed zone, therefore their "shoot zone" apparently acts as a root zone.

Physiological Aspects

Morphological effects. Eshel (26) indicated that alachlor reduced height and prevented lateral root growth of cotton seedlings. Edmondson (25) observed similar effects with cucumber (Cucumis sativus L.). Alachlor severely inhibited cucumber growth when 2 ppmw was applied to dry seeds. When one and one-half day old cucumber seedlings were placed in 5 ppmw of the herbicide, root growth ceased within 12 to 16 hours. Reduced growth of yellow nutsedge foliage and tubers has also been observed (70).

Biochemical effects. In a study to determine the effects of alachlor on several metabolic systems of cucumber, Edmondson (25) reported that fresh weight and incorporation of ^{14}C -leucine into protein of excised hypocotyl sections was stimulated by alachlor. This stimulation of

^{14}C -leucine incorporation was evident even in roots which had been treated long enough to severely inhibit root growth. The RNA and DNA content also increased in root tissue after alachlor treatment. Edmondson observed no effect in ^{32}P -labeling of polyribosomes in root tips nor in ribonuclease and invertase activity. The activity of proteolytic enzymes in the cotyledons of cucumber seedlings were severely inhibited if the plants were germinated in 5 ppmw alachlor, but no effect was noted on these enzymes if pregerminated seedlings were treated with the herbicide.

Devlin and Cunningham (22) showed that alachlor inhibited GA induction of amylolytic activity in embryo-free barley seed halves, but only at the higher concentrations. Alachlor at 3.7×10^{-6} M decreased amylase activity only 3 percent while at 3.7×10^{-4} M, its activity was inhibited 37 percent. Rutledge (70), on the other hand, found that alachlor decreased starch utilization in yellow nutsedge tubers but did not prevent starch hydrolysis by amylase during the nine week period of assessment.

Possible Mode of Action and/or Selectivity

Devlin and Cunningham (22), observed that alachlor was capable of inhibiting GA-induced amylolytic activity in embryo-free barley seed halves. They suggested that part of alachlor's toxicity was due to the inhibited flow of food material from the storage areas of the seed to the growing embryo. They concluded this could severely reduce or terminate a plant's growth.

Eshel (26) did not postulate a mode of action of alachlor but suggested that its selectivity to cotton is based mainly on depth protection and does not result from a physiological degradation in the plant. He further stated that most of the crops which exhibit tolerance to the herbicide have relatively large seed and are sown rather deep.

II. HERBICIDAL-PLANT PROTECTANTS

Hoffman (40) suggested four advantages of antidotes for herbicides. If they were available he proposed that they might allow:

1. The use of higher rates, thus permitting more effective weed control.
2. The use of herbicides under conditions where crop damage is liable to occur such as susceptible varieties, or adverse weather or soil conditions.
3. The use of nonselective herbicides for selective weeding of crops.
4. The protection of valuable plants that have been accidentally treated with a herbicide.

Activated carbon and various other absorbants have been used successfully on alfalfa (Medicago sativa L.) (55), beans (Phaseolus vulgaris L.) (1,2), cabbage (Brassica oleracea L.) (1,2), oats (Avena sativa L.) (2,4), strawberry (Fragaria grandiflora Ehrh.) (1,20,52,56) and tobacco (Nicotiana tabacum L.) (1,2) for protection from various herbicides at ratios of 100 to 400:1 (herbicide:absorbant). Since these are absorbants, they provide protection by physically decreasing the

amount of herbicide absorbed by the plant. This review will consider these compounds only on the basis of their relationship to chemical antidotes.

Foliar Applied Protectants

Among the first compounds, other than absorbants, which exhibited herbicide antidotal properties were the hormone-type herbicides. Pfeiffer et al. (65) observed that the effectiveness of 4-chloro-2-butynyl m-chlorocarbanilate (barban) in controlling wild oats in winter wheat was reduced if 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,3,6-trichlorobenzoic acid (2,3,6-TBA) had been applied to the wheat up to four days prior to barban treatment. Barban activity was also reduced by MCPA (2-methyl-4-chlorophenoxyacetic acid) and mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid]. Further studies of Pfeiffer et al. (65) established that none of these herbicides were antagonistic to barban when applied one week after the barban. Similar results were presented by Hoffman et al. (40) who reported that MCPA could be applied with an equal volume of barban as a tank mix without significantly reducing barban's control of wild oats, however, when larger amounts of MCPA were used, the activity of barban was reduced.

Hoffman (40), postulated that since auxin-type herbicides were antagonistic to the phytotoxic activity of barban, an antiauxin such as 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) should have a sparing action on barban and thus increase its activity. Laboratory studies showed that 2,4,6-T was also an antagonist of barban. Hoffman noted

that although 2,4,6-T does not result in typical auxin plant responses such as a curvature or swelling, it does cause a formative effect on tomato as do the more typical hormone-type herbicides. This led him to conclude that the protective effect was related to the formative processes in the plant.

Seed Treatments as Herbicidal Antidotes

Foliar applied protectants do not appear to be practical for extensive use in crop production because their protective action is nonspecific. Because of this lack of specificity, crop injury can be reduced, but is accompanied by less effective weed control. Hoffman (40) suggested that the most feasible time to use herbicidal antidotes was when the crop is separate from the weeds, such as before the crop seeds are sown. In utilizing this seed treatment concept he compared 46 compounds for barban antagonism on wheat and found that approximately 75 percent of the inhibitory effect of the foliar applied herbicide was eliminated by dusting the seeds before planting with 4'-chloro-2-hydroxyiminoacetanilide, 2,4-dichloro-9-xanthenone and N-methyl-3,4-dichlorobenzene sulfonamide. As observed with the foliar applied antagonist, the protective effect was found in those chemicals which produced formative effects on plants.

In 1969, Gulf Chemical Company presented data at the annual meeting of the Weed Science Society of America which indicated that 1,8-naphthalic anhydride seed treatment could be used to protect corn from otherwise lethal rates of EPTC (41). Other studies substantiated this protective effect for both EPTC (8,10,14,15,16,42,47,49,57,64,66,67,69,83,84) and

another thiocarbamate, S-ethyl diisobutylthiocarbamate (butylate) (14,47,57,69,83,84).

Burnside et al. (16), in summarizing data collected over a two year period on three Nebraska soil types, reported that NA at 0.5 percent by corn seed weight eliminated most of the corn yield loss from EPTC at 3.4, 6.7 and 10.0 kg/ha. The NA also partially reduced the moderate corn yield loss from butylate at 9.0 kg/ha.

The NA has been shown to be effective in increasing the tolerance of corn to alachlor, 3-amino-2,5-dichlorobenzoic acid (chloramben), and S-propylthiocarbamate (vernolate) (57). The average increase in crop tolerance from NA was 1.5 to 2.0X.

Rice plants receiving 0.5 percent NA seed treatment, exhibited less injury from S-ethyl hexahydro-1H-azepine-1-carbothioate (molinate) and S-(4-chlorobenzyl)-N,N-diethylthiocarbamate (IMC-3950) than plants not receiving the seed treatment (38). However, the use of the antidote was found to increase the phytotoxicity of vernolate and EPTC on rice.

III. ALACHLOR-NA INTERACTION

Numerous attempts to use alachlor for weed control in grain sorghum have been reported (18,24,26,29,48,57,72). Results of those studies were inconsistent with extensive variability due to environmental conditions and soil types.

Chenault et al. (18) observed that sorghum was not injured by preemergence applications of alachlor up to 4.48 kg/ha, when rainfall for the first five days after treatment was only 0.025 cm. However,

when 4.45 cm of rain fell during the first five days after application, as little as 0.56 kg/ha of alachlor reduced grain yields 1120 kg/ha.

Somabhi and Pothisoonthorn (73) reported that alachlor increased yields of sorghum on a red clay loam in a wet season, but was toxic to the crop and reduced its germination on a white sandy clay loam. Eshel (26), using sorghum as a bioassay plant, observed a 50 percent reduction in plant growth with alachlor concentrations as low as 1 ppmw on three of four soils tested. The difference among the soils could be related to differences in texture and organic matter content.

Alachlor can be used as a preemergence herbicide in grain sorghum without significantly reducing its yield, if NA is used as a seed treatment (48,57,72). Jordan and Jolliffe (48) presented data showing that sorghum plants grown from seeds treated with 0.5 percent NA were protected from injury caused by alachlor and S-2,3-dichloroallyl N,N-diisopropylthiocarbamate (diallate). Foliage and root weight determinations indicated that the counteracting effect of NA was greater in the shoots than in the roots of grain sorghum.

Shelby (72), using percent plant vigor reduction, plant height, and dry weight of seedlings as indicators, reported that 0.5 percent NA protected grain sorghum from severe alachlor injury. The NA rates of 1.0 and 2.0 percent w/w did not provide significant additional protection above the 0.5 percent rate at alachlor concentrations of 2.24, 3.36, and 6.72 kg/ha.

The manner by which NA protects grain sorghum and corn from herbicide injury has not been conclusively established. Hahn and Merkle

(34,35,36), using a germination bioassay procedure employing disposable growth pouches, evaluated the effectiveness of 0.5 percent NA and various other seed treatments in protecting sorghum from alachlor (16 ppmw) injury. Untreated sorghum showed a 61 percent reduction in shoot growth while the NA treated showed only a 25 percent reduction. Gibberellic acid (GA) and hydrochloric acid (HCl) provided a similar degree of protection showing a 26 and 31 percent reduction in growth. Activated carbon, IAA, naphthaleneacetic acid (NAA), and chloroethylphosphonic acid provided some protection with shoot reductions of 53, 55, 47, and 46 percent respectively. All treatments except NA and activated carbon were made by allowing the sorghum seed to imbibe on filter paper saturated with the solutions for 12 hours.

Hahn and Merkle also observed no differences in the rate of alachlor dissipation from a fine sandy loam soil treated with 1.5 ppmw alachlor and incubated with and without 150 ppmw NA.

These results, indicating that NA was not acting as an absorbant and was not chemically breaking down the alachlor molecule led them to conclude that NA could be reacting with alachlor in the vicinity of the germinating seed and rendering it less phytotoxic.

Holm et al. (43), in studying the metabolism of the experimental herbicide 2,5-dimethyl-1-pyrrolidine carboxanilide in corn, concluded that NA acts by inducing corn seedlings to metabolize more of the herbicide during the early seedling stage which is most susceptible to injury.

CHAPTER III

MATERIALS AND METHODS

A preliminary study was conducted to determine if the protective action afforded by NA to alachlor treated grain sorghum and EPTC treated corn could be reproduced in a vermiculite-nutrient solution growth medium under greenhouse conditions. Results of this study showed that the injurious action of the herbicide and the protective effect of NA were more easily detectable with the alachlor-NA interaction in grain sorghum than with the EPTC-NA interaction in corn. Because of this increased detectability, the alachlor-NA interaction was selected for subsequent studies.

The ensuing investigations consisted of two distinct phases, the first being morphological and physiological studies involving experiments in which attempts were made to locate the site of action of alachlor and of the protective action of NA. Results of these studies indicated that one of the primary sites of alachlor action was in the emergence of the primary leaf from the coleoptile. Thus, the second phase of the study was concerned with specific physiological and biochemical studies in the coleoptiles of corn. Corn, rather than grain sorghum, was used for these studies because it would exhibit alachlor injury as well as the protective NA effect while providing more plant material for analysis.

I. PRELIMINARY STUDY

The experiment was established on March 3, 1971 in a greenhouse at Knoxville, Tennessee. Each treatment consisted of planting either 15 grain sorghum or 15 corn seeds treated with 0, 0.5, 1, or 5 percent (w/w) NA in 10 cm diameter plastic pots which were filled with vermiculite. Alachlor at rates of 0, 1, 5, or 25 ppmw was applied to pots containing grain sorghum and EPTC at rates of 0, 2, 8, or 32 ppmw was applied to pots containing corn. Herbicides were applied in solution with Hoagland's #2 nutrient solution (39), and concentrations maintained by keeping the pots watered to a constant weight. At the end of the second week, additional nutrient solution was applied to the germination media.

Average plant height and visual vigor reduction ratings were determined 31 days after planting. For the visual rating, a zero to ten scale was used in which zero indicated no reduction in plant vigor and ten indicated that the plants were dead.

The experimental designs were four by four factorial arrangement of treatments in randomized complete block design with five replications.

II. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES

WITH GRAIN SORGHUM

Germination Experiments

Fifty grain sorghum seeds, treated with 0 or 2 percent NA (w/w), were germinated in 100 mm disposable petri dishes on Whatman's #1 filter paper moistened with 20 ml of 0, 1, 5, or 25 ppmw alachlor. The experiments were conducted at 30 C in the dark (some exposure to light while

data were being recorded). Coleorhiza and coleoptile emergence counts were made 24, 48, and 72 hours after treatment.

The experimental design was a two by four factorial arrangement of treatments in a randomized complete block design with five replications. The experiment was repeated three times.

Primary Leaf Emergence Study

Twenty grain sorghum seeds treated with 0 or 2 percent NA were placed in 13.5 x 13.0 cm plastic trays on filter paper moistened with 0, 1, 2, 3, 4, or 5 ppmw alachlor. The trays were placed in a dark incubator and maintained at 30 C. The number of plants exhibiting normal primary leaf emergence from the coleoptile and the shoot length (mesocotyl + coleoptile) of each plant were recorded after five days. The experimental design was a two by five factorial arrangement of treatments in a randomized complete block design with four replications. The experiment was duplicated.

Effective NA Concentration in Solution

This experiment was conducted to determine if NA would provide protection to the grain sorghum plant if applied as a solution, and if so, what concentration was the most effective without being detrimental to the plants. Twenty-five grain sorghum seeds were planted into a vermiculite medium in 10 cm diameter plastic pots. The treatments consisted of applying 250 ml of Hoagland #2 nutrient solution containing 0, 5, 50, 150, or 250 ppmw NA in combination with either 0, 1, 5, or 25 ppmw alachlor. The solution must be kept stirred while applying in

order to maintain even distribution of NA. The pots were watered to volume every other day. The plants were grown in a growth chamber at 30 C and 90 percent RH in the dark (some exposure to light during the watering process). The experimental design was a four by five factorial arrangement of treatments in a randomized complete block design with five replications.

Seven days after planting a visual rating was made as described in the preliminary study. At the same time, the percentage of coleoptiles exhibiting normal leaf emergence from the coleoptile were recorded as another indicator of herbicidal effect. The seven-day period was selected for measurements because it was thought that a longer dark period would reduce vigor of the control plants.

Leaf Distortion Study

Fifteen grain sorghum seeds were planted in 10 cm diameter plastic pots containing vermiculite saturated with 0 or 150 ppmw NA in combination with 0, 1, 2, 3, 4, or 5 ppmw alachlor. The 150 ppmw NA concentration was used because it provided sufficient protection from alachlor in the study above with the least vigor reduction among the various concentrations tested (0, 5, 50, 150, and 250 ppmw NA).

The pots were maintained in the dark at 30 C. After seven days, the plants were watered with nutrient solution and placed under a 12 hours light, 12 hours dark photoregime. Fifteen days after planting, the plants in each treatment were classified visually into four categories of leaf distortion: normal, slight, moderate, and severe (Figure 1).

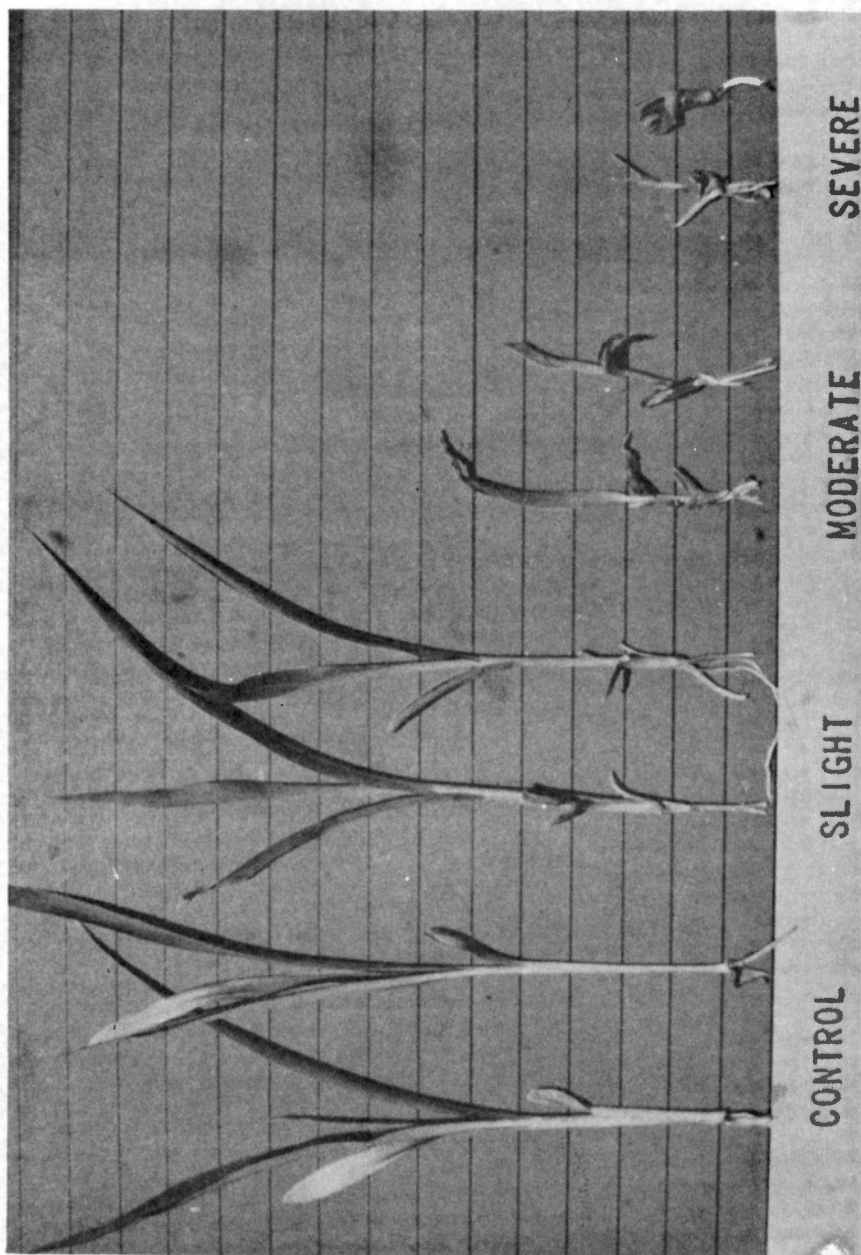


Figure 1. Visual leaf distortion categories of 16-day-old grain sorghum seedlings caused by preemergence treatment with alachlor.

Four weeks after planting, the number of plants in each treatment with normal leaves was recorded.

The experimental design was a two by six factorial arrangement of treatments in a randomized complete block design with four replications. The experiment was conducted twice.

Primary Leaf Emergence and Unrolling Study

Approximately 200 grain sorghum seeds were planted in vermiculite moistened with 10^{-4} M CaCl_2 or 10^{-4} M CaCl_2 + 150 ppmw NA and grown in the dark at 30 C for four days. At the end of four days, before any primary leaves had emerged from coleoptiles of either treatment, the terminal 3.5 cm segments of 120 plants of both treatments were removed and placed in distilled water. Twenty segments of each treatment were transferred to 100 mm petri dishes containing 25 ml of 0.001 M NaOAc pH 5.5 buffer solution with 0, 1, 5, 15, 25, or 50 ppmw alachlor, covered with a piece of filter paper and vacuum infiltrated for 15 minutes at 1.76 kg/cm^2 in a vacuum oven. The segments were then transferred to a 75 by 25 mm glass slide wrapped with filter paper and placed in a petri dish containing 10 ml of the solution with which the segments had been infiltrated. Aeration was provided by holes made in the petri dish tops. At the end of 72 hours, the width of the first leaf was determined by projecting the image with an overhead projector and measuring the projected image. The image was magnified 10X.

The experimental design was a two by six factorial arrangement of treatments in a randomized complete block design with four replications. The experiment was duplicated.

III. PHYSIOLOGICAL AND BIOCHEMICAL STUDIES

WITH CORN COLEOPTILES

Vermiculite was the growth medium used in these studies and all chemicals were applied in 10^{-4} M CaCl_2 . Plants were grown in the dark at 30 C until analyzed. Chemical concentrations were maintained by keeping pots watered to a constant weight.

Hormone Studies: GA

Ten seeds were planted in plastic pots and treated with either: control (10^{-4} M CaCl_2), 150 ppmw NA, 20 ppmw alachlor, 150 ppmw NA + 20 ppmw alachlor, 50 ppmw GA (75 percent potassium salt), 50 ppmw GA + 20 ppmw alachlor, or 50 ppmw GA + 150 ppmw NA.

Seven days after planting, the number of coleoptiles exhibiting normal leaf emergence and length of the coleoptiles were recorded. The experimental design was a randomized complete block with four replications. The experiment was duplicated.

Hormone Studies: IAA

Corn was planted as in the GA experiment except chemical treatments were: control, 150 ppmw NA, 20 ppmw alachlor, or 150 ppmw NA + 20 ppmw alachlor. On the fifth day after planting, the coleoptiles were harvested, leaves removed, and a 1 cm section was cut from each coleoptile with a double edge cutter, 5 mm from the tip. The sections were incubated on a medium as described by Nooden et al. (60) for IAA-induced cell elongation studies. The medium contained 2 percent sucrose, 0.1 mM

penicillin GK and 5 mM pH 5.0 potassium phosphate buffer. The incubation medium was brought to a final concentration of 0 or 10 ppmw IAA. Five coleoptiles from each treatment were placed in separate 50 ml erlenmeyer flasks containing 25 ml of the incubation medium with and without IAA. Sections were incubated for 20 hours at 30 C on a shaker in the dark prior to measurement on an overhead projector (10X). The experimental design was a randomized complete block design with four replications. The experiment was duplicated.

Leaf Retention Study

Approximately 150 seeds were planted in aluminum trays and treated with 10^{-4} M CaCl_2 , 150 ppmw NA, or 25 ppmw alachlor with and without 150 ppmw NA. On the fifth day after planting, ten coleoptiles were removed at the coleoptilar node from each treatment and the maximum force required to induce slippage of the leaves from the coleoptile was determined with a constant-rate-of-extension instrument (Instron Universal Testing Instrument).

Coleoptiles were prepared for analysis by first removing the apical tip to expose the top 5 mm of the enclosed leaves. Injury to the leaves was minimized by longitudinally slitting the 5 mm section of the coleoptile to be removed into halves or quarters and removing the segments with tweezers. Then at the basal end, the coleoptile was slit longitudinally just above the node and a 5 mm section of the leaf teased out with tweezers, severed from the remaining portion of the leaf still enclosed within the coleoptile and removed. No allowances

were made for differences in length of coleoptiles since the force required to induce slippage of the leaf from the coleoptile would be directly related to the point of maximum resistance throughout the coleoptile. Coleoptiles thus prepared were placed upright in standard #2 clamps with masking tape fitted on the faces of the grips. With the upper clamp (holding the leaf) fixed, the lower clamp (holding the coleoptile) was then traversed down at a constant rate of 0.127 cm/min, increasing the load on the coleoptile. The maximum force required to induce slippage, in grams, was recorded. The experimental design was a two by two factorial arrangement of treatments in a randomized complete block with four replications. Due to the time required to conduct the experiment, replications were conducted on different days.

IAA Effect on Alachlor-Induced Leaf Retention

Five coleoptiles were excised with the leaves intact from plants grown in the presence of 25 ppmw alachlor, length measured, and placed in a 100 mm petri dish containing 20 ml of 0 or 10 ppmw IAA elongation solution (60) and placed in the dark at 30 C for 16 hours. At the end of this incubation period, the length of the coleoptiles was again measured to determine percent elongation and prepared for leaf retention analysis as outlined above. The experimental design was a randomized complete block with four replications. The experiment was duplicated.

RNA-DNA Ratio

One hundred coleoptiles were harvested from plants grown as described in the leaf retention study, the leaves removed and the sections frozen

until analyzed. The procedure used for isolation and identification of the nucleic acids was that of Cherry et al. (19). The experimental design was a two by two factorial arrangement of treatment in a randomized complete block with four replications. Replications were conducted on different days.

Chemical Constituents

Approximately 250 coleoptiles were harvested from plants grown as described in the leaf retention study. The enclosed leaves were removed and the coleoptiles placed in water until all leaves had been removed. After removing the bulk of the water by suction, the coleoptiles were blotted on paper towels, and fresh weight determined. The samples were dried at 62 C and reweighed to obtain percent dry matter before being ground in a Wiley mill equipped with a 20-mesh sieve and stored for analysis.

Cellulose, hemicellulose, and acid-detergent lignin were determined using the methods described by Van Soest (80) for forage fiber analysis. Percent protein of the samples was determined by placing 0.200 g of ground sample in concentrated sulfuric acid and allowing it to digest overnight. The mixture was then heated to over 200 C for 2.5 hours. At the end of this period, 15 ml of 35 percent hydrogen peroxide were added and the mixture reheated until clear and then for 45 minutes after clearing. Following heating, the solution was placed in a 250 ml volumetric flask and brought to volume with distilled water. From this 250 ml, an aliquot was placed in an Auto-Analyser (78) and percent

transmission determined after the phenol-hypochlorite color reaction (79). From this transmission value, the percent protein-nitrogen was calculated. Percent protein was taken as 6.25 times the percent protein-nitrogen content.

The experimental design was a two by two factorial arrangement of treatments in a randomized complete block with four replications. Due to the time required to collect plant material, replications were harvested on different days.

Statistical Analysis

Comparison of treatments were made where indicated by use of Duncan's Multiple Range Test. Treatments within each test followed by the same letter are not significantly different at the 5 percent level of probability.

CHAPTER IV

RESULTS

I. PRELIMINARY STUDY: ALACHLOR-NA INTERACTION IN GRAIN SORGHUM

Significant differences in plant height and vigor were observed with various alachlor and NA concentrations (Tables 3 and 4). As a general trend, increasing alachlor concentrations significantly reduced plant height regardless of NA treatment. This trend was consistent over all treatments except with 1 ppmw alachlor concentration in combination with 1 and 5 percent (w/w) NA. Plant height was reduced more severely by all alachlor treatments when NA was not present than when it was present. Increased NA concentrations beyond the 0.5 percent level (1 and 5 percent w/w) did not result in significantly taller plants than the lowest concentration when in combination with 1, 5, or 25 ppmw alachlor. Plant height was also reduced by increasing NA concentration in the absence of alachlor.

One, five, and twenty-five ppmw alachlor significantly reduced plant vigor, with 5 and 25 ppmw reducing vigor more than 1 ppmw. Vigor of plants treated with 1 ppmw alachlor in combination with 0.5, 1, and 5 percent NA was not significantly lower than the control. The NA treated plants had less reduction in vigor at the 5 and 25 ppmw alachlor rates than non-NA treated plants. The 5 percent NA treatments in the absence of alachlor reduced plant vigor below that of the control and

TABLE 3

GRAIN SORGHUM HEIGHT 31 DAYS AFTER TREATMENT WITH
ALACHLOR AND NA SEED TREATMENT

Alachlor ppmw	Percent NA (w/w)			
	0	0.5	1	5
	-----cm-----			
0	9.0 a*	8.5 b	7.8 c	6.6 d
1	5.6 e	7.8 c	7.7 c	7.8 c
5	0.7 g	6.0 e	5.6 e	6.1 e
25	0.2 h	3.9 f	3.7 f	3.7 f

*Numbers followed by the same letter are not significantly different at 0.05 level of probability using Duncan's Multiple Range Test.

TABLE 4

VIGOR REDUCTION RATINGS OF GRAIN SORGHUM 31 DAYS AFTER
TREATMENT WITH ALACHLOR AND NA SEED TREATMENT

Alachlor ppmw	Percent NA (w/w)			
	0	0.5	1	5
	-----rating†-----			
0	0.7 g*	0.4 g	1.2 g	3.2 def
1	5.6 bc	0.4 g	1.6 fg	2.0 defg
5	8.6 a	4.0 cd	4.4 bcd	3.6 de
25	9.6 a	6.0 b	6.0 b	6.0 b

*Numbers followed by the same letter are not significantly different at 0.05 level of probability using Duncan's Multiple Range Test.

†0 indicates no reduction; 10 indicates death of the plant.

the 0.5 and 1 percent NA concentrations. The NA concentrations of 1 and 5 percent did not result in significantly more vigorous plants than the lowest concentration (0.5 percent) when applied in combination with alachlor.

II. PRELIMINARY STUDY: EPTC-NA INTERACTION IN CORN

Results similar to those obtained with the alachlor-NA interaction in grain sorghum were observed with the EPTC-NA interaction in corn (Tables 5 and 6). The EPTC concentrations greater than 2 ppmw resulted in reduced plant height and plant vigor if NA was not used as a seed treatment. The NA levels of 0.5 and 1 percent (w/w) NA did not significantly reduce plant height nor plant vigor below that of the controls. A reduction of these parameters was, however, observed with the 5 percent NA level.

Neither 2 nor 8 ppmw EPTC in combination with 0.5, 1, or 5 percent NA significantly reduced plant height or plant vigor below that of the respective NA treatment alone. However, plants grown in the presence of 32 ppmw EPTC in combination with these NA levels were significantly shorter than plants grown in the presence of NA only. This was also true for plant vigor ratings at the 0.5 and 1 percent NA levels, but 32 ppmw EPTC in combination with 5 percent NA did not significantly reduce plant vigor below that of the 5 percent NA alone treatment. The 0.5 percent NA level was as effective as the 1 and 5 percent level in protecting corn from EPTC induced height and vigor reductions.

TABLE 5
CORN HEIGHT 31 DAYS AFTER TREATMENT WITH EPTC
AND NA SEED TREATMENT

EPTC ppmw	Percent NA (w/w)			
	0	0.5	1	5
	-----cm-----			
0	16.3 a*	14.1 abc	15.3 ab	13.2 bcd
2	14.2 abc	12.5 cde	13.3 bcd	13.0 bcde
8	8.3 g	12.6 cde	13.0 bcde	11.4 def
32	5.6 h	10.6 ef	11.1 def	10.1 fg

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

TABLE 6

VIGOR REDUCTION RATINGS OF CORN 31 DAYS AFTER TREATMENT
WITH EPTC AND NA SEED TREATMENT

EPTC ppmw	Percent NA (w/w)			
	0	0.5	1	5
	-----rating†-----			
0	1.2 fg*	1.6 efg	0.8 g	3.2 bcde
2	2.4 defg	2.8 cdef	1.6 efg	2.8 cdef
8	6.8 a	3.2 bcde	2.8 cdef	2.8 cdef
32	7.2 a	3.6 bcd	4.4 bc	4.8 b

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

†0 indicates no reduction; 10 indicates death of the plant.

III. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES WITH GRAIN SORGHUM

Germination Experiments

Coleorhiza emergence through the pericarp was not affected by 1, 5, or 25 ppmw alachlor in the presence or absence of 2 percent NA, or by NA alone, at 24, 48, or 72 hours after treatment (Table 7).

No coleoptiles had emerged 24 hours after treatment. With 48 hours of treatment, 2 percent NA reduced the number of coleoptiles emerging when compared to the control. Two percent NA in combination with 1 and 5 ppmw alachlor did not decrease coleoptile emergence below that of the two alachlor treatments alone. The NA-alachlor treatment however, decreased emergence below that of the alachlor treatment alone at the 25 ppmw herbicide concentration (Table 8).

The NA effect on coleoptile emergence observed 48 hours after treatment was still evident after 72 hours except neither the 5 nor 25 ppmw alachlor treatments alone reduced coleoptile emergence as much as when applied in combination with 2 percent NA.

Primary Leaf Emergence Study

In the germination study, the leaves of some grain sorghum plants grown for at least five days in darkness in the presence of alachlor did not emerge from the coleoptile, or if emergence did occur, it was along the sides of the coleoptile and not through the tip as occurred in nonalachlor treated plants or alachlor treated plants receiving 2 percent NA seed treatment (Figure 2).

TABLE 7

COLEORHIZA EMERGENCE OF 50 GRAIN SORGHUM SEEDS 24, 48, and 72 HOURS
AFTER TREATMENT WITH ALACHLOR AND NA SEED TREATMENT*

Alachlor ppmw	24 Hours		48 Hours		72 Hours	
	0 NA	2 Percent w/w NA	0 NA	2 Percent w/w NA	0 NA	2 Percent w/w NA
	-----number of coleorhiza emerging-----					
0	48	48	48	48	49	49
1	48	48	49	48	49	49
5	48	47	48	48	49	49
25	47	47	48	48	49	49

*Numbers within each treatment period were not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

TABLE 8

COLEOPTILE EMERGENCE OF 50 GRAIN SORGHUM SEEDS 48 AND 72 HOURS
AFTER TREATMENT WITH ALACHLOR AND NA SEED TREATMENT

Alachlor ppmw	48 Hours		72 Hours	
	0 NA	2 Percent w/w NA	0 NA	2 Percent w/w NA
	-----number of coleoptiles emerging-----			
0	48 a*	47 b	49 a	48 b
1	48 a	47 b	49 a	48 b
5	48 a	47 b	48 b	47 c
25	48 a	46 c	48 b	47 c

*Numbers within each treatment period followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

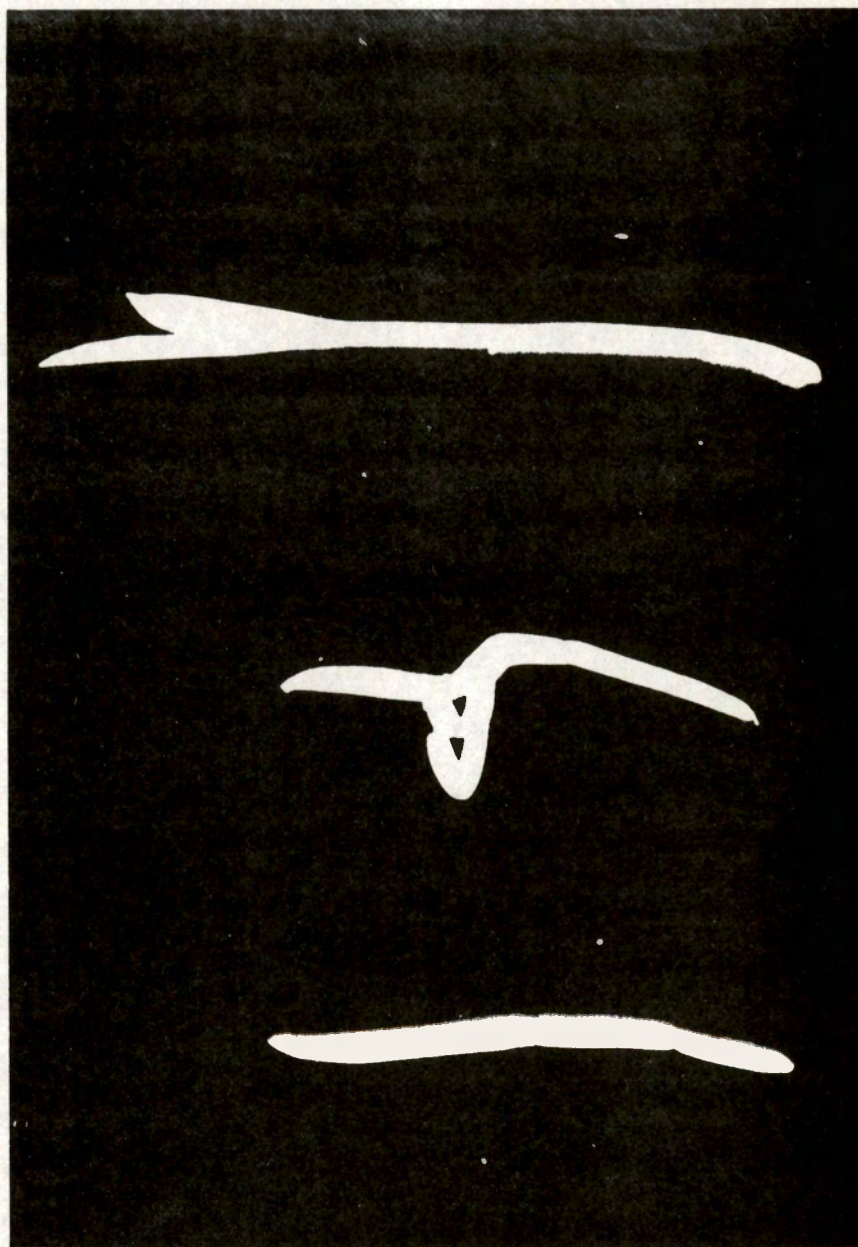


Figure 2. Effects of alachlor on emergence of primary leaves from grain sorghum coleoptiles. Alachlor treated plants exhibiting no leaf emergence (L), or abnormal emergence through the sides of the coleoptile (C). The plant on the right is a control plant.

In subsequent studies all alachlor treatments reduced normal leaf emergence below that of the controls ($\pm$ 2 percent NA). All alachlor treatments, except 1 ppmw, inhibited primary leaf emergence from the coleoptile less when 2 percent NA was present (Table 9).

The failure of primary leaves to emerge normally through the coleoptile was not simply an overall growth reduction response. Plants grown in the presence of 3, 4, and 5 ppmw alachlor in combination with 2 percent NA were not significantly taller than plants receiving the same herbicide treatment without NA. A significantly higher percentage of plants exhibited normal leaf emergence at these herbicide concentrations when NA was used as a seed treatment (Table 9, Figure 3).

Effective NA Concentration in Solution

Plant vigor and percent of plants exhibiting normal leaf emergence were significantly reduced by 5 and 25 ppmw alachlor regardless of NA treatment (Table 10). Both vigor ratings and percent normal leaf emergence decreased as alachlor concentration increased across all NA treatments, except the vigor of plants grown in the presence of 5 ppmw alachlor in combination with 150 ppmw NA was not significantly lower than plants grown in 1 ppmw alachlor in combination with 150 ppmw NA.

The 150 ppmw NA concentration was selected for all subsequent studies because it gave the highest plant vigor rating of all NA concentrations tested when applied in combination with 5 ppmw alachlor (although differences were not significant), and was equivalent to the 250 ppmw NA concentration in the percent of plants exhibiting normal leaf emergence.

TABLE 9

SHOOT HEIGHT AND PERCENT NORMAL LEAF EMERGENCE OF DARK GROWN
GRAIN SORGHUM SEEDLINGS FIVE DAYS AFTER TREATMENT
WITH ALACHLOR AND NA SEED TREATMENT

Alachlor ppmw	Height		Normal Emergence†	
	0 NA	2 Percent w/w NA	0 NA	2 Percent w/w NA
	-----cm-----		-----percent-----	
0	7.9 a*	5.2 c	73.8 a	70.0 ab
1	7.4 a	5.0 cd	53.8 cd	61.3 bc
2	6.6 b	4.7 cde	38.8 f	50.6 de
3	4.5 def	4.0 efg	4.4 g	41.9 ef
4	4.0 efg	3.9 fg	1.9 g	36.9 f
5	2.9 h	3.5 gh	0.6 g	43.8 ef

*Numbers within measurement categories followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

†Percent of germinated plants exhibiting normal leaf emergence from the coleoptile.

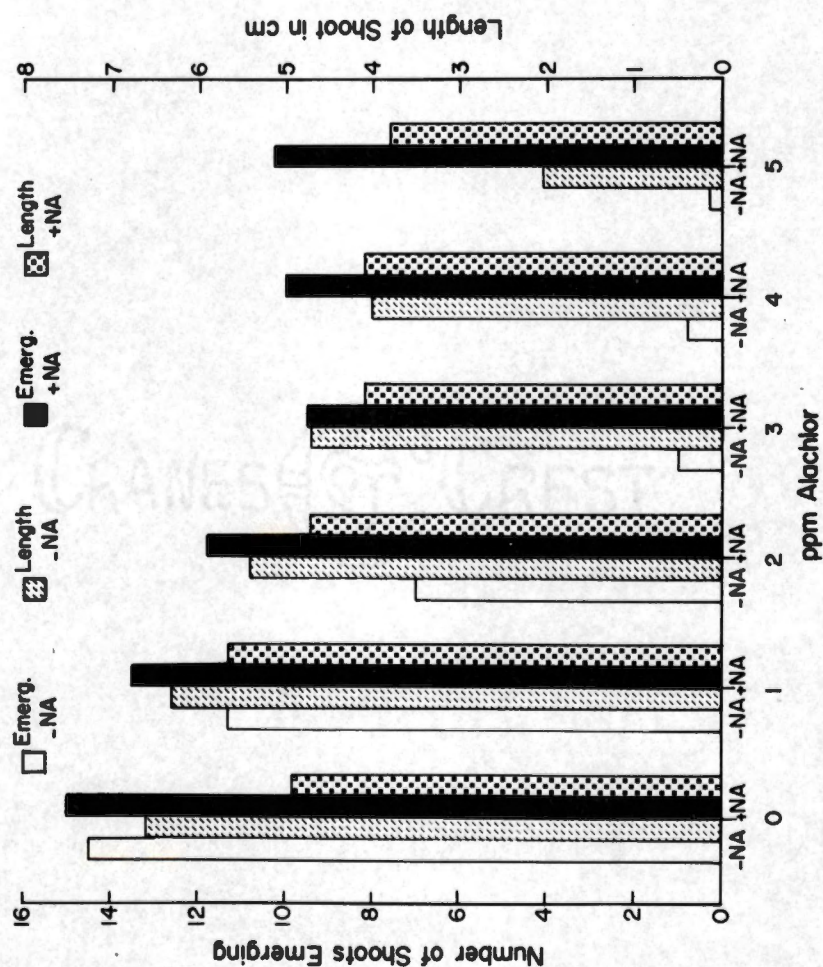


Figure 3. Effect of alachlor and NA (applied as seed treatment) on shoot height and percent normal leaf emergence of dark grown sorghum seedlings.

TABLE 10

VIGOR REDUCTION RATING AND PERCENT NORMAL LEAF EMERGENCE OF DARK GROWN GRAIN
SORGHUM SEEDLINGS SEVEN DAYS AFTER TREATMENT WITH ALACHLOR AND
VARIOUS NA CONCENTRATIONS APPLIED AS SOLUTION

Alachlor ppmw	0 NA		5 ppmw NA		50 ppmw NA		150 ppmw NA		250 ppmw NA	
	Rating	Emer. %†	Rating	Emer. %	Rating	Emer. %	Rating	Emer. %	Rating	Emer. %
0	0.0 a*	97 a	0.0 a	99 a	0.2 a	99 a	1.7 b	98 a	2.2 bc	100 a
1	0.5 a	96 a	0.5 a	98 a	0.5 a	96 a	2.0 bc	99 a	2.5 bcd	97 a
5	3.5 def	3 d	4.2 f	56 c	3.5 def	67 bc	3.0 cde	70 bc	3.7 ef	80 b
25	6.2 g	0 d	6.5 gh	0 d	6.0 g	0 d	7.0 gh	0 d	7.5 h	0 d

*Numbers within measurement categories followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

†Percent of germinated plants exhibiting normal leaf emergence from the coleoptile.

Leaf Distortion Study

Various degrees of leaf distortion resulted when alachlor and alachlor-NA treated grain sorghum plants, grown for seven days in vermiculite in the dark, were placed in light for nine days (Figure 1, page 20). Analysis of the data showed that all alachlor treatments without 150 ppmw NA resulted in more plants exhibiting either slight, moderate, or severe distortion than the controls, the NA treatment, or any alachlor treatment in the presence of NA (Table 11). Alachlor concentrations of 3, 4, and 5 ppmw without NA resulted in significantly more plants with severe leaf distortion than any other treatment. Plant response to 1 and 2 ppmw alachlor was intermediate between 5 ppmw and the control. More plants exhibited slight leaf distortion with 1 ppmw alachlor and 5 ppmw alachlor + NA than to other treatments. One and two ppmw alachlor without NA also resulted in more plants exhibiting moderate leaf distortion than other treatments. A summary of these data is shown in Figure 4. The number of coleoptiles exhibiting each type of distortion is expressed as percentage of population of all coleoptiles emerging through the vermiculite. For example, with 1 ppmw alachlor approximately 4 percent of the coleoptiles had leaves with no distortion, 20 percent had slightly distorted leaves, 36 percent had moderately distorted leaves, while all others had severely distorted leaves (40 percent).

Primary Leaf Emergence and Unrolling Study

In all alachlor treatments greater than 1 ppmw, the degree of primary leaf emergence and unrolling (expressed as leaf width in mm 10X)

TABLE 11

VISUAL LEAF DISTORTION OF GRAIN SORGHUM SEEDLINGS GERMINATED AND GROWN IN THE PRESENCE OF ALACHLOR AND NA IN THE DARK FOR SEVEN DAYS, THEN SUBJECTED TO A 12-HOUR LIGHT 12-HOUR DARK PHOTOREGIME FOR EIGHT DAYS

Alachlor ppmw	Visual Distortion Category†											
	None			Slight			Moderate			Severe		
	0 NA	150 ppmw	NA	0 NA	150 ppmw	NA	0 NA	150 ppmw	NA	0 NA	150 ppmw	NA
0	100.0 a*†	100.0 a		0.0 b	0.0 b		0.0 b	0.0 b		0.0 d	0.0 d	
1	3.9 d	100.0 a		19.9 a	0.0 b		37.1 a	0.0 b		38.5 c	0.0 d	
2	0.0 d	100.0 a		6.8 b	0.0 b		31.1 a	0.0 b		62.1 b	0.0 d	
3	0.0 d	90.4 ab		4.3 b	9.6 b		10.0 b	0.0 b		85.6 a	0.0 d	
4	0.0 d	86.6 b		0.0 b	9.4 b		7.9 b	1.4 b		91.3 a	1.6 d	
5	0.0 d	65.4 c		0.0 b	20.1 a		4.5 b	4.6 b		94.5 a	10.8 d	

*Numbers within categories followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

†Expressed as percent of emerged seedlings exhibiting defined distortion.

‡See Figure 2, page 36.

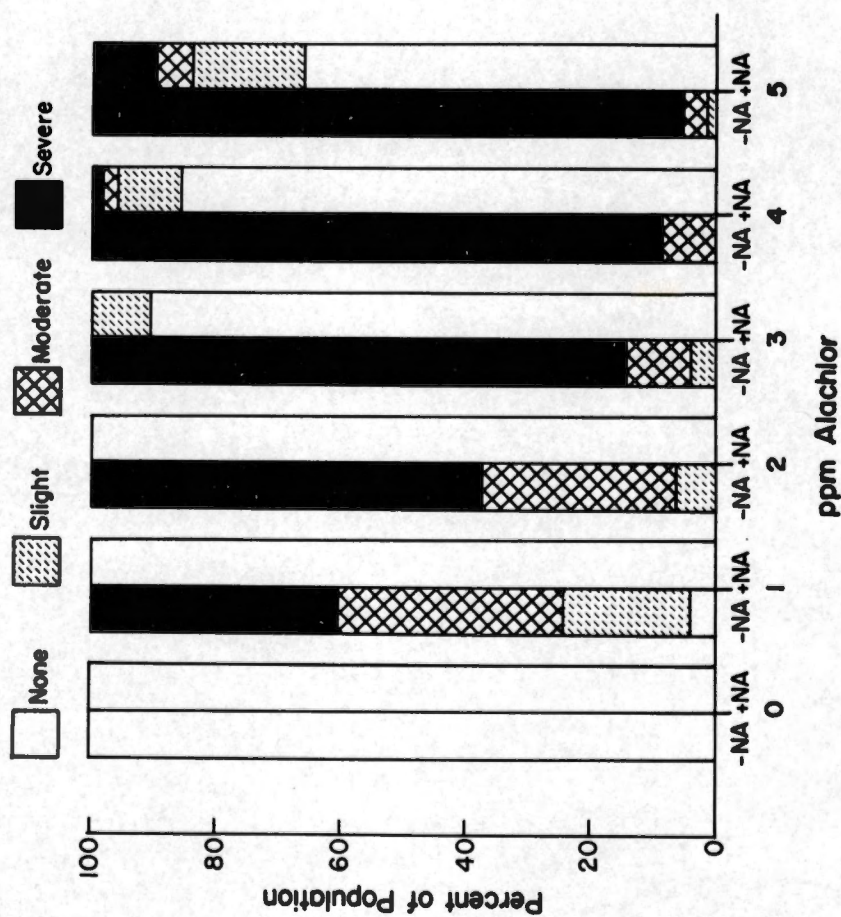


Figure 4. Effects of alachlor and 150 ppmw NA (applied as solution on visual leaf distortion of grain sorghum seedlings). The number of coleoptiles exhibiting each type of distortion is expressed as percentage of population of all coleoptiles emerging through the vermiculite.

of excised terminal segments was less than that of nonalachlor treated segments (Table 12). This was true regardless of whether the segments were from plants grown in the presence of 150 ppmw NA or not. Increasing alachlor concentrations greater than 5 ppmw reduced primary leaf width more than the preceding lower concentration (e.g., 15 < 25 < 50), regardless of NA treatment. Width of leaves of segments from plants grown in NA were wider than those not grown in NA when vacuum infiltrated with 15, 25, or 50 ppmw alachlor.

When the coleoptile was removed from the segments vacuum infiltrated with 50 ppmw alachlor and the primary leaf unrolled, leaf width was not significantly lower than that of the controls regardless of whether or not the segments were from NA treated plants or not.

IV. PHYSIOLOGICAL AND BIOCHEMICAL STUDIES WITH CORN COLEOPTILES

Hormone Studies: GA

Coleoptiles of plants treated with 50 ppmw GA were longer than those in all other treatments including the controls. Both 150 ppmw NA and 20 ppmw alachlor alone and in combination had shorter coleoptiles than the control plants, with the 150 ppmw NA + 20 ppmw alachlor combination resulting in plants with shorter coleoptiles than all other treatments. The GA treatment completely reversed the shortening effect caused by 150 ppmw NA, but only partially overcame that of the 20 ppmw alachlor treatment (Table 13).

TABLE 12

PRIMARY LEAF EMERGENCE AND UNROLLING OF EXCISED TERMINAL
SEGMENTS OF GRAIN SORGHUM SEEDLINGS GROWN IN THE
PRESENCE OF 0 OR 150 ppmw NA SOLUTION THEN
VACUUM INFILTRATED WITH ALACHLOR

Alachlor ppmw	0 NA mm	150 ppmw NA mm
0	4.8 a	4.7 a
1	4.6 ab	4.7 a
5	4.3 bc	4.3 c
15	2.6 e	4.2 c
25	1.9 f	3.6 d
50	1.6 g	2.9 e
50†	4.7 a	4.7 a

*Numbers followed by the same letter are not significantly different at 0.05 level of probability using Duncan's Multiple Range Test.

†50 ppmw alachlor, coleoptile removed and leaf unrolled.

TABLE 13

LENGTH AND PERCENT NORMAL LEAF EMERGENCE OF CORN COLEOPTILES
AFTER TREATMENT WITH ALACHLOR, NA, AND GA

Treatment	Length mm	Percent of Control	Normal Emergence percent
Control	61.1 b*	---	100.0 a
150 ppmw NA	51.4 cd	84	100.0 a
20 ppmw alachlor	49.5 d	81	44.9 b
50 ppmw GA	69.7 a	114	100.0 a
50 ppmw GA + 150 ppmw NA	61.1 b	---	100.0 a
50 ppmw GA + 20 ppmw alachlor	55.6 c	91	97.4 a
150 ppmw NA + 20 ppmw alachlor	43.8 e	72	100.0 a

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

As observed in earlier preparatory studies, less than 50 percent of the plants treated with 20 ppmw alachlor exhibited normal primary leaf emergence. This alachlor-induced reduction was completely counteracted by the 150 ppmw NA treatment and the 50 ppmw GA treatment (Table 13).

Hormone Studies: IAA

The 1 cm coleoptile segments from plants grown in the presence of 150 ppmw NA, 20 ppmw alachlor, and 150 ppmw NA + 20 ppmw alachlor responded more to IAA-induced elongation than did the segments from the control plants. Segments from plants grown in 20 ppmw alachlor and 150 ppmw NA + 20 ppmw alachlor responded more than those from the 150 ppmw NA treatments (Table 14).

Leaf Retention Study

More force was required to induce leaf slippage in coleoptiles from plants grown in the presence of 25 ppmw alachlor than in any other treatment. When 150 ppmw NA was applied in combination with alachlor the force required was not different from the control. The NA treatment alone reduced the force required to induce leaf slippage below that of other treatments including the controls (Table 15).

The values obtained for the alachlor treatment are not absolute because in many instances the force required to induce slippage of the leaves within the coleoptile was greater than the tensile strength of the leaves, and in other cases the coleoptile would slip from the clamps before slippage of the leaves occurred. These difficulties were not encountered with any other treatment.

TABLE 14

PERCENT IAA-INDUCED ELONGATION OF 1 cm CORN COLEOPTILE SEGMENTS
FROM PLANTS TREATED WITH ALACHLOR AND NA

Treatment	Percent Increase by IAA†
Control	17.3 c*
150 ppmw NA	31.4 b
20 ppmw alachlor	40.8 a
150 ppmw NA + 20 ppmw alachlor	41.3 a

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

†10 ppmw IAA.

TABLE 15

FORCE REQUIRED TO INDUCE LEAF SLIPPAGE IN CORN COLEOPTILES
AFTER TREATMENT WITH ALACHLOR AND NA

Treatment	Force g
Control	21.6 b*
150 ppmw NA	7.1 c
25 ppmw alachlor	55.4 a
150 ppmw NA + 25 ppmw alachlor	13.2 bc

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

IAA Effect on Alachlor-Induced Leaf Retention

The length of coleoptiles from plants grown in the presence of 25 ppmw alachlor increased 43 percent when floated for 16 hours in 10 ppmw IAA as compared to a 13 percent increase when IAA was not added to the elongation medium. This increased elongation however did not decrease the amount of force required to induce leaf slippage in the coleoptiles of these plants (Table 16).

RNA-DNA Ratio

Neither 150 ppmw NA nor 25 ppmw alachlor significantly altered the RNA-DNA ratio in the coleoptiles of corn below that of the control. This ratio was increased by the combination of these two treatments (Table 17) however. The RNA-DNA ratio of plants grown in the presence of 150 ppmw NA was intermediate between the control and alachlor-NA combination treatments.

Chemical Constituents

Twenty-five ppmw alachlor affected all constituents measured in this study except percent hemicellulose (Table 18). Percent dry matter was highest in coleoptiles from alachlor treated plants and lowest in those from control plants. The 150 ppmw NA treatment increased percent dry matter of the coleoptiles with the alachlor-NA treatment being intermediate between the 150 ppmw NA treatment and the 25 ppmw alachlor treatment. Dry matter content per 100 coleoptiles, however was higher than the controls only in the 25 ppmw alachlor treatment.

TABLE 16

FORCE REQUIRED TO INDUCE LEAF SLIPPAGE IN CORN COLEOPTILES
GROWN IN THE PRESENCE OF 25 ppmw ALACHLOR
AFTER IAA TREATMENT

Treatment	Percent Elongation After 16 Hours	Force g
-IAA	13	49.6 a*
+IAA†	43	43.8 a

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

†Coleoptiles incubated for 16 hours in the dark in elongation solution $\pm$ 10 ppmw IAA.

TABLE 17
RNA-DNA RATIO OF CORN COLEOPTILES AFTER TREATMENT
WITH ALACHLOR AND NA

Treatment	RNA-DNA Ratio
Control	42.1 b*
150 ppmw NA	49.7 ab
25 ppmw alachlor	43.2 b
150 ppmw NA + 25 ppmw alachlor	67.9 a

*Numbers followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

TABLE 18

CHEMICAL CONSTITUENTS OF CORN COLEOPTILES AFTER TREATMENT WITH ALACHLOR AND NA

Treatment	DM g	DM/100 plants g	Cell. g	Hemi. g	Lig. g	Protein g
Control	4.55 d*	0.98 b	14.3 a	29.9 a	1.83 a	17.7 c
150 ppmw NA	4.72 c	0.89 b	13.8 a	28.5 a	1.48 ab	16.2 c
25 ppmw alachlor	6.31 a	1.33 a	11.5 b	31.0 a	0.95 b	23.7 a
150 ppmw NA + 25 ppmw alachlor	5.22 b	0.96 b	13.4 a	28.8 a	0.33 c	19.7 b

*Numbers within a column followed by the same letter are not significantly different at the 0.05 level of probability using Duncan's Multiple Range Test.

Alachlor decreased percent cellulose and lignin below that of the controls. Percent cellulose was not altered by 150 ppmw NA, but when NA was applied in combination with 25 ppmw alachlor, the alachlor-induced reduction was nullified. The NA applied in combination with alachlor did not nullify the alachlor-induced reduction of lignin as it did cellulose, but acted synergistically and further reduced lignin content below that of all other treatments.

The alachlor-NA interaction was also evident in the protein content of corn coleoptiles. Percent protein was highest in coleoptiles from plants grown in 25 ppmw alachlor. The NA applied in combination with alachlor decreased this stimulation of protein content but not completely, i.e., protein content of coleoptiles from these plants was intermediate between values obtained for control plants and those obtained for the 25 ppmw alachlor treatment. Hemicellulose content was not affected by any of the treatments.

CHAPTER V

DISCUSSION

The fact that alachlor is a preemergence soil incorporated herbicide is suggestive that its primary mode of action is exerted or expressed during the early stages of plant development. Likewise, the protective effect afforded by NA would also have to be exerted at this early stage of growth.

Results of these studies indicate that grain sorghum germination was not significantly delayed by alachlor, whereas normal seedling development was impaired. Devlin and Cunningham (22) reported that alachlor inhibited GA-induced amylase activity in embryo-free barley (Hordeum vulgare L.) seed halves by 37 percent. They suggested that alachlor may interfere with the flow of food material from the storage areas of the seed to the growing embryo resulting in reduced plant growth. Rutledge (70), on the other hand, concluded that alachlor decreased starch utilization, but did not prevent starch hydrolysis by amylase in yellow nutsedge tubers.

The effect of alachlor on emergence of the primary leaf from the coleoptile does not appear to be a result of interference with translocation or utilization of stored food reserves since plants treated with 3, 4, or 5 ppmw alachlor and receiving NA seed treatment were not significantly taller than those treated with 3, 4, or 5 ppmw alachlor without the protectant and which exhibited herbicide injury.

The much elongated mesocotyl and short coleoptile, which are characteristic of dark grown grain sorghum plants, make it difficult to establish whether normal leaf emergence and development is the result of retention of the leaves within the coleoptile or a direct inhibition of leaf unrolling by alachlor. The former appears to be the case since subsequently emerging secondary leaves not enclosed in the coleoptile appear to unroll normally.

Results obtained from the primary leaf emergence and unrolling study also suggest a retention by the coleoptile. The primary leaves partially emerged from segments vacuum infiltrated with 15, 25, and 50 ppmw alachlor, but complete unfolding appeared to be restricted by the coleoptile. When the coleoptile was removed and the leaf unrolled, leaf width was not significantly lower than the controls.

The twofold increase in the amount of force required to induce leaf slippage in corn coleoptiles from plants grown in the presence of 25 ppmw alachlor is even more conclusive that the coleoptile is in some manner inhibiting normal emergence of the primary leaf. The alachlor-NA interaction was also expressed in the leaf retention study. The force required to induce leaf slippage in coleoptiles from plants grown in the presence of the alachlor-NA combination treatment was not different from that obtained for the control. The effect of the 150 ppmw NA treatment on reducing the amount of force required to induce leaf slippage is indicative that it may in some way act by exhibiting a "loosening" effect on the cell walls in the coleoptile.

This observation, in conjunction with the known roles of IAA and GA (85) in the expansion and cell wall loosening properties of Avena coleoptiles is rather suggestive that alachlor may be interfering with the action of these hormones in the coleoptiles of grain sorghum and corn. This interrelationship was researched to some extent by Hahn and Merkle (34,35,36), who reported that GA was twice as effective as IAA in reducing alachlor injury. These results do not constitute conclusive evidence that alachlor is affecting the role of GA more than it is the role of IAA. In their study, they used shoot length of intact plants as an indicator of herbicidal effect and intact plants do not respond to exogenously applied IAA as much as they do to exogenously applied GA. One explanation for this observation is that auxins, either endogenous or exogenously applied, move predominantly basipetal (downward in the phloem) away from the more meristematic region once an optimal level has been reached in this region. Gibberellins, on the other hand, may be continuously moving both basipetally and acropetally (upward in the xylem) (85). When coleoptile segments were excised from plants grown in the presence of alachlor, as in this investigation, and floated in 10 ppmw IAA, they responded more to IAA than did the controls, indicating a possible effect of alachlor on the synthesis or translocation of IAA in the intact plant.

Data of the "effect of IAA on alachlor-induced leaf retention" study, indicate that if alachlor is affecting the role of IAA in the coleoptile interfering with normal leaf emergence, it is independent of an elongation response. Coleoptiles of plants grown in the presence of

25 ppmw alachlor and floated in 10 ppmw IAA elongated 30 percent more than those not floated in IAA, but the force required to induce leaf slippage was not reduced.

Hahn and Merkle (36) reported that 50 ppmw GA provided protection to grain sorghum from alachlor equalivalent to 0.5 percent NA. This observation, coupled with the growth stimulating properties of GA (63), suggest that GA's protection may be attributed to increased leaf growth and more rapid emergence, even though the leaves were retained by the coleoptile. This however was not the case as corn plants treated with 50 ppmw GA + 20 ppmw alachlor exhibited normal emergence from the coleoptile.

The retention of the primary leaves within coleoptiles of alachlor treated plants could lead one to speculate that certain structural polysaccharides within the coleoptiles are not being metabolised. This could result in a more rigid coleoptile or a zone of more rigid cells, and the elongating leaf may not grow through this zone and therefore be retained within the coleoptile. Attempts to measure rigidity of the coleoptiles using the Instron Universal Testing Instrument were unsuccessful, as most coleoptiles would slip from the grips before they would break. Various materials were tested for effectiveness in preventing slippage, but none was satisfactory.

Percent dry matter and dry matter per 100 plants determinations also suggest either an increase in the rate of synthesis or a decrease in the rate of decomposition of a structural component of the coleoptile, as both of these increased in coleoptiles of plants grown in the

presence of 25 ppmw alachlor. However, an analysis of three of the more prevalent structural components of plant cells, cellulose, hemicellulose, and lignin, indicated, contrarily, that cellulose and lignin content decreased in alachlor-treated plants, whereas hemicellulose content was not affected by alachlor treatment.

The increased protein content observed in coleoptiles from plants grown in the presence of 25 ppmw alachlor suggests that a structural protein may be involved in this retention. However, the current knowledge of the role of structural proteins in determining cell wall characteristics does not unequivocally support this hypothesis. Lamport (53) suggested that the hydroxyproline-rich primary cell wall protein, extensin, could cross link wall polysaccharides thus providing a chemical basis for changes in cell wall plasticity. He later presented data (54) suggesting that minute quantities of the amino acid hydroxyproline could form a cross link between two polysaccharide chains resulting in increased cell wall rigidity. Olson et al. (61), on the other hand, using the Instron Universal Testing Instrument to measure mechanical properties of Avena coleoptiles showed that removal of most of the protein in the coleoptiles, including some hydroxyproline-rich protein did not affect cell wall extensibility. However, he did state that the hydroxyproline-rich fraction not removed by this treatment could be structurally important. If such a small quantity of extensin does result in a more rigid cell wall as proposed by Olson et al., quantitative determinations to relate cell wall rigidity to extensin content would not be feasible.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Results of preliminary greenhouse studies showed that the protective effect of NA was more easily detected with the alachlor-NA interaction in grain sorghum than with the EPTC-NA interaction in corn in a vermiculite-nutrient solution growth medium.

Ensuing growth chamber and laboratory studies were conducted to determine the site of action of the protective effect of NA and to study the effects of alachlor in the presence and absence of NA on certain morphological, physiological, and biochemical processes in grain sorghum and corn seedlings.

In germination studies it was determined that alachlor did not affect time of coleorhiza or coleoptile emergence through the pericarp of grain sorghum. However, if alachlor treated plants were maintained in the dark for at least five days, the leaves of some grain sorghum plants did not emerge through the coleoptile, or if emergence did occur, it was along the sides of the coleoptile and not through the tip as occurred in nontreated plants or alachlor treated plants receiving NA seed treatment. This retention within or by the coleoptile resulted in various degrees of leaf distortion when plants grown in the dark for seven days were placed in light for nine days. Severity of distortion increased with increasing alachlor concentration, while the addition of NA reduced severity of distortion. Primary leaves of grain sorghum segments vacuum infiltrated with alachlor did not emerge from the

coleoptile as completely as did segments from nonalachlor treated plants as indicated by leaf width measurements.

The GA completely counteracted NA-induced length reduction observed in corn coleoptiles but was only partially effective in counteracting the alachlor-induced reduction. The NA acted synergistically with alachlor and further reduced coleoptile length. Both NA and GA nullified the alachlor-induced abnormal leaf emergence from the coleoptile. These observations suggest that the roles of GA and NA in counteracting alachlor's effect on corn seedling development were not identical.

The increased force required to induce leaf slippage in corn coleoptiles grown in the presence of alachlor was not sufficiently explained by differences in the structural components, cellulose, hemicellulose, and lignin. Percent protein increased in coleoptiles grown in the presence of alachlor; however, there is little evidence available which would explain leaf retention on the basis of increased protein content. The increased force required to induce leaf slippage was also shown not to be related to cell extensibility as the force required to induce leaf slippage in coleoptiles subjected to IAA-induced elongation was not lower than that obtained for control coleoptiles.

In conclusion, it appears that one of the primary sites of alachlor and NA action and interaction is in the development of the plant during early seedling growth, i.e., leaf emergence from the coleoptile. This study was not adequately able to relate the physiological and biochemical parameters to the observed morphological effect of abnormal leaf emergence from the coleoptile.



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