PHYSIOLOGICAL ASPECTS OF THE HERBICIDAL ACTIVITY OF ALACHLOR

By

JAMES MICHAEL CHANDLER

Bachelor of Science
West Texas State University
Canyon, Texas
1965

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1968

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Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Herbicides have proven to be valuable tools for the control of undesirable vegetation in plants used for food, fiber and aesthetic purposes. As the demand for higher quality and quantity of agricultural products increases the utilization of herbicides will be employed to achieve this goal. Along with this demand for greater efficiency is a rising awareness of the response of our environment to the various factors used to manipulate it for man's good.

Most herbicides used in crop production schemes are selective and since selectivity implies that all plants do not respond the same to all herbicides, it is very important to know the degree of susceptibility of both crop and weed species. An understanding of the basic principles involved in selectivity of a given herbicide could ultimately result in wider and safer utilization of the compound.

The purpose of this study was to gain a better understanding of the herbicidal activity and selectivity of alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide]. The present knowledge of the basis of selectivity of alachlor is very limited. Therefore, an understanding of the uptake and translocation of alachlor could possibly aid in determining the herbicidal selectivity of alachlor. Field, greenhouse, growth chamber and laboratory studies were conducted to accomplish the above purpose.

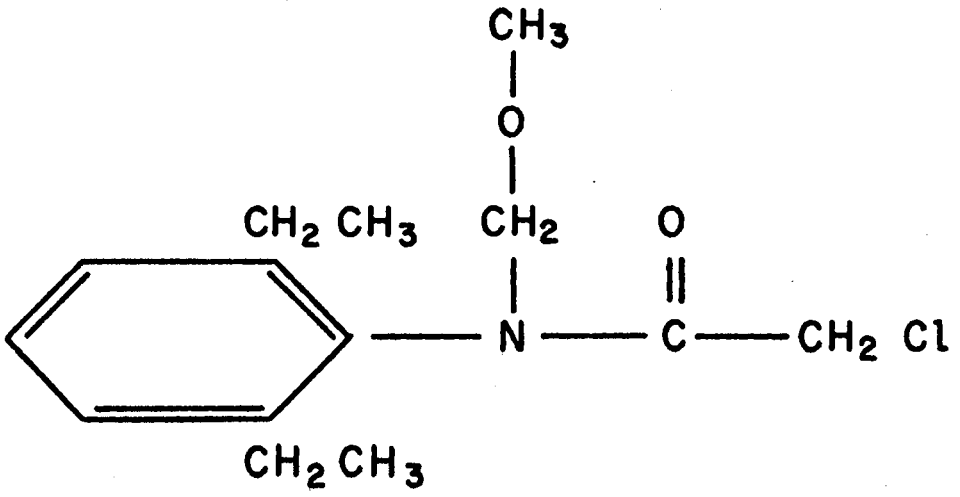
CHAPTER II

REVIEW OF LITERATURE

Chloroacetamides

Herbicidal Properties

Alachlor, a selective preemergence herbicide of the α-chloroacetamide family, is used in Oklahoma for the control of many annual grasses and certain broadleaf weeds in corn (*Zea mays* L.), peanuts (*Arachis hypogaea* L.), and soybeans [*Glycine max* (L.) Merrill]. Alachlor has the following structural formula:



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

Figure 1. Chemical Structure of Alachlor

Ilnicki (19) reported that alachlor appears to be a fairly stable herbicide. Little or no loss occurs through volatilization or photodecomposition. Alachlor appears to be activated under a wide range of soil moisture conditions but more moisture is needed to activate it than for the related propachlor (2-chloro-N-isopropylacetanilide). Alachlor is less susceptible to leaching, than other related herbidices. Generally, it is dissipated within 6 to 8 weeks under field conditions. He also reported that shallow soil incorporation of alachlor 0.5 to 1 inch deep increases activity, particularly where moisture is low.

Knake et al. (26) reported that shallow incorporation did not significantly affect results with propachlor under any moisture condition. Grandal (14) reported no benefit from incorporation of alachlor when compared with surface applications.

The chloroacetamide family of herbicides was first introduced by Monsanto Chemical Company in 1954 (16). The α-chloroacetamides are similar to chlorpropham (isopropyl m-chlorocarbanilate) in that they are both toxic to germinating seeds and lethal to seedlings in a very early stage of development. In contrast to α-chloroacetamides, the urea and triazine herbicide families affect plants at a much later stage of development.

Chloroacetamide herbicides are synthesized by reacting amines with chloracetyl chloride. In primary screening studies the acetamide was found to be superior to the propionamide or the butyramide. Studies in which bromine or iodine were used, in place of the alpha-chlorine substitution, showed chlorine to have the highest activity (6).

While the all-or-none activity seems related to the alpha-halogen atom and the acetamide is superior to propionamide or butyramide, the

relative activity is modified very much by the remainder of the molecule, the particular amine used in forming the compound.

The three carbon chain length and the benzene ring with certain substitutions gives a high level of herbicidal activity. There may be a size or configuration relationship to the absorption or penetration of the molecule into the germinating seedling.

Degradation

Jaworski (21) has shown that crop plants normally treated with CDAA (N,N-diallyl-2-chloroacetamide) or CDEC (2-chloroallyl diethyldithiocarbamate) are capable of metabolizing the molecules to naturally occurring metabolic products. In corn the chloroacetic portion of CDAA was converted to glycolic acid. The allylic portions were changed as measured by the evolution of $^{14}CO_2$ from the middle carbon of the allylic radical. The metabolism of the 2-chloroallyl moiety of ^{14}C-labled CD EC indicates that cabbage was capable of liberating the 2-carbon of the allyl moiety as $^{14}CO_2$. One of the primary metabolic breakdown products of the 2-chloroallyl grouping was lactic acid. He concluded that probably the rapid metabolic degradation leads to the high degree of resistance shown by the crop species.

Soil dissipation studies showed the half-life of CDAA to be 16 to 18 days, which was fairly representative over a broad range of soil types and climatic conditions (22). Some of the loss could be attributable to volatility (8).

Metabolism of uniformly ring-labeled ^{3}H-propachlor in corn and soybeans was extremely rapid with no tritiated propachlor at the earliest harvest in 3 days (24). All the radioactivity in the plants

remained in a soluble form and was not fixed into polymeric, insoluble products.

The partitioning characteristics of the corn metabolite indicate that more than 90% of the metabolite was water soluble. Vapor-phase chromatographic analysis of an ether extract from acid hydrolysis of the corn metabolite fortified with cold 2-hydroxy-N-isopropylacetanilide as a carrier indicated that the major radioactive peak coincided with the hydroxy analog (24). Studies with soybeans showed similar results (22).

Propachlor is metabolized to a water soluble acidic metabolite but the absolute structure is not known. It is known that the metabolite contains essentially the entire structure of the original herbicide, with the exception that the chloro group appears to have been displaced, probably by some nucleophilic endogenous substrate in the plant (22).

According to Smith et al. (37) the selectivity of α-chloroacetamides is based on differential rates of metabolism between sensitive and resistant plant species (37). The metabolism of eight 2-chloroacetamides were considered in oat (Avena sativa L.) and cucumber (Cucumis sativis L.) seeds, moderately susceptible species, and corn and soybeans, highly resistant species. The degree of metabolism was determined at 6 and 48 hours. At 6 hours there was a definite difference between the degree of metabolism by corn and by oats. Corn metabolized significant amounts within a short time, but oats, the more susceptible species, metabolized essentially none.

The same relationship was observed between soybeans and cucumbers. Both species metabolized large fractions in 48 hours, but only the

resistant soybeans metabolized a significant amount in 6 hours.

The degree of susceptibility of seeds of various species to chloroacetamides could be directly related to the length of time required to initiate metabolism of these chemicals.

Uptake

The importance of shoot entry in the action of herbicides was discussed and reviewed by Parker (35). He showed that CDEC and CDAA had more inhibitory effect on shoots than on roots. Knake and Wax (26) found that propachlor was just as effective in the shoot zone as in the seed zone while alachlor was more effective in the shoot zone than in the seed zone. Both propachlor and alachlor caused a reduction in the number of plants when placed in the shoot zone.

Both corn and soybean plants were capable of rapid uptake of radioactive propachlor from treated soil (24).

Studies by Smith et al. (37) using several α-chloroacetamides showed two types of uptake curves depending on the plant species used. Corn, soybean and oats data showed a parabolic uptake curve while the uptake curve for cucumbers was sigmoid and was the same for all derivatives. Corn, one of the more resistant species, took up less chemical than the other seeds, but soybeans, the other resistant species took up more than any other seed or about three times that of corn. Since oats and cucumbers are susceptible and corn and soybeans very resistant, it appears from Smith's studies that the susceptibility is not determined by the amount of chemical absorbed.

Mode of Action

Jaworski (20) in 1956 reported the effects of CDAA upon respiration of germinating wheat (Triticum vulgara Vill.) and ryegrass (Lolium perenne L.) seeds. Ryegrass respiration was strongly inhibited by CDAA, whereas it was only moderately affected in germinating wheat, a much less susceptible plant species. The data suggested that CDAA could inhibit certain sulfhydryl containing enzymes involved in respiration but the basic lethal effect must involve some mechanism more intimately connected with growth.

The ability of chloroacetamides to inhibit crystalline α-amylase hydrolysis of soluble starch was studied (23) and neither propachlor nor CDAA inhibited α-amylase at 10^{-3}M. The chloroacetamides might interfere with the gibberellic acid induced amylase production. Both propachlor and CDAA caused significant inhibition of GA_3-induced α-amylase production at concentrations of 10^{-4}M. The inhibitory response was assumed to represent an effect on protein synthesis or some molecular level preceding protein synthesis, since the effect of CDAA was not reversed at high levels of GA_3.

Mann et al. (30) surveyed a large variety of herbicides and their effect upon protein synthesis. In these studies barley coleoptiles or Sesbania hypocotyls were preincubated with CDAA for 1 hour. L-leucine-1-^{14}C was then added and incubated for an additional 2 hours. The tissue was extracted with hot ethanol, and residual ^{14}C was assayed by liquid scintillation counting. A substantial inhibition of leucine incorporation into protein resulted. Mann concluded that the inhibition of protein synthesis by CDAA is probably due to a more fundamental action, since even the uptake of amino acids was somewhat inhibited by

CDAA.

Work conducted by Dhillon et al. (9) showed that propachlor inhibits protein and lipid breakdown in squash cotyledons during germination and early seedling growth. In other studies (9) it was found that propachlor inhibited the activation of amino acids both in vivo and in vitro in the case of squash, a susceptible species, and only in vitro in corn, a resistant species.

In studies with cucumber seedlings, Duke (10) indicates that propachlor inhibited all expansion induced by 2,4-D in hypocotyl tissue and at a similar concentration inhibited protein synthesis. The inhibition of auxin induced cell expansion was attributed to the prevention of auxin induced enzyme formation. The inhibitory effect of propachlor on the growth of cucumber roots was closely correlated with the inhibition of protein synthesis in root tips. Protein synthesis was significantly reduced before root growth became inhibited. The inhibition of protein synthesis by propachlor was not associated with an effect on ATP formation or on respiration. Propachlor only slightly affected RNA synthesis but drastically reduced nascent protein formation. The inhibition of nascent protein formation was thought to be due to the prevention of transfer of amino-acyl-s-RNA to the growing polypeptide chain. It was concluded that the mode of action of propachlor is based on its effect on protein synthesis.

Jaworski (23) suggested that because of the reactive nature of the α-halogen in the α-chloroacetamides, a nucleophilic displacement may occur between the amino group of methionyl-RNA and herbicide. If such a reaction were to occur with methionyl-RNA, in which the transfer, RNA, was specifically for the initiation step, then an interference

with the mechanism of protein initiation could occur.

The present knowledge on the mode of action of alachlor is very limited. Baird and Upchurch (2) found that the response of six grass species as influenced by temperature to alachlor could not be predicted on their classification as warm or cool season grasses.

Studies conducted in Oregon (14) showed alachlor was more toxic to barnyardgrass seedlings when the coleoptile emerged through treated soil than when the roots grew into treated soil. Dissipation studies, determined that the rate of detoxification was strongly dependent on the temperature of incubation and on the rate of alachlor applied. Maximum detoxification occured at 25 C with 2 ppm alachlor broken down after 59 days of incubation.

Edmondson, (11) using cucumbers as the test species showed that seedlings treated with either alachlor or propachlor are visually undistinguishable from each other. Inhibition of root growth by alachlor and propachlor was similar with the exception that alachlor treated roots increased more in fresh weight after root length had been severely inhibited. Excised cucumber hypocotyl sections cultured with alachlor gained more fresh weight than the control sections.

Excised tissue cultured in alachlor resulted in growth stimulation similar to that produced by an auxin herbicide. However, if exogenous auxin was added to the culture solution, alachlor acted in an antagonistic manner by partially inhibiting the 2,4-D induced growth.

Alachlor caused a stimulation of ^{14}C-leucine incorporation into root protein. Polyribosome formation was not affected by alachlor while the amount of RNA and DNA in root tissue of treated cucumbers was increased. Alachlor caused a 68.6% reduction in proteinase

activity in cucumber cotyledons while little effect on ribonuclease activity in root tip sections was unaffected by either alachlor or propachlor.

Photosynthesis (Hill Reaction)

In 1937 Hill (17) reported that isolated chloroplast or chloroplast fragments would evolve oxygen under the influence of light in the presence of a suitable hydrogen acceptor upon photolytic cleavage of water. This photolytic reaction became known as the Hill reaction. Arnon (1) states that this reaction can be considered as "photosynthesis with a substitute oxidant" or photosynthesis without carbon dioxide fixation.

Wessels and van der Veen (40) were the first to show that monuron [3-(p-chlorophenyl)-1,1-dimethylurea], a substituted phenylurea, inhibited the Hill reaction of isolated chloroplast.

The inhibitory effects of various alkyl N-phenylcarbamates on the Hill reaction of isolated turnip green (Brassica spp.) chloroplast were investigated by Moreland and Hill (34). They found in general that carbamates possessing a free imino hydrogen atom were highly active as Hill reaction inhibitors while a substitution of this hydrogen by an alkyl or aryl group resulted in a loss in, or decrease of, inhibitory power. Found in this group of compounds are two important herbicides, propham (isopropyl carbanilate) and chlorpropham, which gave 50% inhibition of the Hill reaction at concentrations of 1.2×10^{-4} M and 2.9×10^{-4} M respectively. They postulate that the carbonates may act by indiscriminately covering the surface of the chloroplyll-protein complex in the Hill reaction or through specific absorption at an active site. They go on to state that the chemical behavior of this

group of compounds and the response shown in the chloroplast system are such as to eliminate the involvement of ionic or covalent bonds. The carbamates can be removed from the chloroplast by washing and photolytic activity is subsequently restored. The binding forces involved are more than likely relatively weak. Hence, hydrogen bonding and van der Waals electrostatic attraction forces are possibly involved. The atoms of the alkyl N-phenylcarbamate molecule that can take part in hydrogen bond formation are the imino hydrogen, carbonyl oxygen, and the ring-substituted chlorine atoms. Moreland and Hill (34) thinks that the imino hydrogen plays an important role in the hydrogen bond formation reaction. It may take part in hydrogen bond formation with some electronegative constituent located either at or near the reactive center in the chloroplasts. Evidence in support of this suggestion is derived from two sources: compounds in which the imino hydrogen is replaced are not potent inhibitors of the photochemical reaction and derivatives with chlorine substituted at an ortho position of the benzene ring lack inhibitory properties. The chlorine in the ortho position may be able to form a hydrogen bond intramolecularly - chelate ring formation - with the imino hydrogen, or through steric influence it could prevent an electronegative group from approaching close enough to the imino hydrogen to form a hydrogen bond. From their studies Moreland and Hill (34) did not know whether the ringsubstituted chlorine played a direct or an indirect role in inhibiting the photochemical reaction.

Good (13) investigated more than 200 acylanilides, thiocylanilides, acylamides, ureas and thioureas as potential Hill reaction inhibitors. He states that the only feature common to all the Hill reaction

inhibitors investigated is the presence of an imino hydrogen. To extend the work done by Wessels and van der Veen (40) and Moreland (34) on the relationship of inhibitor structure to inhibitor potency Good states that there is good reason to believe that the imino hydrogen must not only be present but also accessible if a substance is to have significant inhibitory action. In ortho-chloroanilides, the bulky acyl group almost certainly lie in the plane of the benzene ring and remote from the also bulky chlorine atom. Such an orientation would force the imino hydrogen into close proximity with the chlorine atom where it would be shielded from interactions with adjacent molecules. Consequently ortho-chloroanilides may be expected to, and do, resemble N-methyl-anilides in having low melting points, low biological activity, and no hydrogen bonding. In contrast, the imino hydrogen of 2,6-dichloroanilines would be forced into a position away from the plane of the benzene ring and midway between the two chlorines. In this position the imino hydrogen should be, and apparently is, available for hydrogen bond formation and for whatever other function may be involved in producing the inhibition.

Good states that Wessel's suggestion that the imino hydrogens of phenylureas from hydrogen bonds with the carbonyl oxygen of the cyclopentanone ring of chlorophyll, encounters two serious objections. In the first place such a mechanism of inhibition should equally affect photosynthesis, photoreduction, the Hill reaction with ferricyanide or FMN, and the Hill reaction with PMS. He goes on to state that this is not the case. In the second place he shows that the better inhibitors are much too effective. In summary Good says that substitution of the hydrogen on the 3-, 4- or 5- positions of the benzene ring of the

aniline derivatives by Cl, Br, CH_3O or CH_3 increases the inhibitory effect. Substitution of the ortho position practically abolishes inhibition. He goes on to say that chloracetyl-N-methylaniline, an anilide which lacks an imino hydrogen atom, is scarcely inhibitory. Finally Good says that attempts to relate inhibitor potency to the bonding tendency of the imino hydrogen were only partially successful; the rate of hydrogen bonding in the mechanism of inhibition remains uncertain.

Moreland and Hill (32) reported that the sensitivity of the Hill reaction to various derivatives within a chemical family, in many cases, seems to be closely related to the established behavior of the compounds both in the plant and in the soil. At least alteration to the molecular structure which tends to strengthen the herbicidal properties are also associated with an increased inhibition of the Hill reaction.

The effect of mono- and dichlorophenyl analogs of N-phenyl-2-methyl-pentanamide (PMP) and N-(3,4-dichlorophenyl)-alkylamides on the photolytic activity of chloroplast isolated from turnip greens was studied by Moreland and Hill (33). They found that PMP inhibited the Hill reaction by 50 percent at 4.5×10^{-5} M. Monochlorination in the meta and para ring position enhanced the inhibitory activity whereas chlorination in the ortho position negated inhibitory activity of the parent chemical. Replacement of the imino hydrogen of 3,4-DC PMP with a methyl group reduced inhibitory activity below that of the unsubstituted parent compound of PMP.

In their discussion Moreland and Hill (33) suggested some ways through which N-phenylcarbamates, substituted phenylureas and acylanilides could interfere with the Hill reaction. These all involve the

hydrogen-bonding capacities of the imino hydrogen and carbonyl oxygen. First they say that the photochemical reaction energy is considered to be transferred from carotenoids and other pigments to chlorophyll, and also from chlorophyll molecule to chlorophyll molecule. Such transfer and pooling of energy is required to provide sufficient quanta to effect a Hill reaction. Consequently every chlorophyll molecule does not take part in the evolution of oxygen. Suggestions that energy may be pooled by several hundred chlorophyll molecules have been made. How this migration of excitation energy occurs is not known, but the whole chlorophyll-protein complex is thought to be involved.

The inhibitors could alter the secondary and tertiary structure of the chlorophyll-protein by hydrogen bonding with free imino hydrogen and carbonyl oxygen atoms of the constituent protein molecules. A consequence of this action would be a change in structure and configuration of the chlorophyll-protein which would affect its function in the transfer of energy.

Secondly a theory proposed to explain the transmission of energy through proteins involves proton shifts (3). A proton shift type of transfer may be involved in the channeling of photochemically produced "reducing power". A hydrogen-bridge structure across polypeptide chains are involved and the removal of an imino hydrogen atom shifts the bridged groups from the keto to the enol form. The energy difference between the keto and enol forms of the system is considered to be in the order of 1 kcal/mole.

Moreland and Hill (32) say that many workers still consider that the site of the photochemical function is the cyclopentanone ring of chlorophyll despite what Good thinks of the system. They say the

inhibitor could hydrogen bond through carbons 9 and 10 in the cyclopentanone ring of chlorophyll. The transfer of excitation energy from chlorophyll to an acceptor would be prevented by this association. The energy acceptor has not been identified but Wessels proposes that vitamin K might serve in this capacity.

Nitrate Reductase and Associated Constituents

The enzymatic reduction of nitrate to nitrite has been known for about 65 years and conclusive evidence for the existence of nitrate reductase has been known for 18 years. Nitrate reductase extracted from wheat leaves has an optimum activity at pH 7.4 and at a temperature of 29 C.

The enzyme nitrate reductase is induced by its substrate, nitrate (5). However, the enzyme requires not only nitrate but also light (15) and CO_2 (25). It has been suggested that the requirement for light was due to the dependence of the reducing system on reduced nocotinamide-denine dinucleotide (NADPH). The CO_2 requirement for induction of nitrate reductase was believed to be due to dependence on a photosynthetic product for the synthesis of the enzyme. It was recently shown that carbon dioxide allows the reduction of nitrate in vivo through its participation in the synthesis of malic acid, which is necessary to avoid extreme pH changes in the tissue (28).

Lips and Roth-Bejerano (28) showed that suitable concentrations of kinetin and gibberellic acid permit induction of nitrate reductase in leaves of tobacco in the dark. They therefore concluded that light is necessary for nitrate reductase induction or synthesis in leaves of tobacco because of its effect on the concentration of one or more

endogenous growth regulators.

Frank and Grigsby (12) studied fourteen weedy species that were treated with six different herbicides for possible changes in the nitrate content. They found only two species where the accumulation of toxic concentrations of nitrate could be attributed solely to the effect of herbicidal treatment. These two weeds were Eupatorium maculatum and Impatiens biflora.

Beevers et al. (4) using cell-free extracts prepared from corn and cucumber plants sprayed with varying levels of 2,4-D showed that the level of nitrate reductase was increased in corn and reduced in cucumbers. They postulated that 2,4-D could possibly cause intramolecular changes in protein sulfhydryl groups which could result in a modification of enzyme structure. This modification of structure may result in enhanced activity as in corn or an inhibition as in cucumbers.

In both peach and apple trees more growth and higher leaf nitrogen resulted from application of simazine plus amitrol-T than from supplemental nitrogen treatments, indicating that the herbicides influenced the nitrogen metabolism of these trees (36).

Tweedy and Ries (38) found that non-toxic levels of simazine added to the root zone of corn plants grown under both sub-optimal temperatures and low nitrate levels, increased the nitrogen content and dry weight of plants by 20 to 25%. The increase was found to be associated with an effect on nitrate reductase and was attributed to simazine causing an increase in either nitrate absorption, nitrate assimilation or both.

Reversal studies on the inhibition of respiration suggest that the haloacetamides can react with sulfhydryl reagents (39). Since it is

thought that nitrate reductase has a sulfhydryl group on the active enzyme surface there exists the possibility of an interaction between the enzyme and alachlor.

CHAPTER III

MATERIALS AND METHODS

Primary Screening Studies

Field Study

To evaluate possible preemergence uses of alachlor on several crop and weed species, a field study was conducted on the Oklahoma State University Agronomy farm at Stillwater, Oklahoma. The study was conducted as a factorial arranged in a randomized block design, with 4 applications. The plant species were planted with a tractor-planter in rows 10 inches apart. The application of alachlor was made with an experimental plot sprayer mounted on a cub tractor which applied 30 gallons of water per acre. The alachlor was applied in 40 inch bands at right angles to the rows of plant species at rates of 0.25, 0.5, 0.75, 1, 2, 4 and 8 pounds per acre (lb/A).

The following table will describe the environmental conditions existing at the time of treatment:

Environmental Conditions	Data
Air Temperature	39 C
Soil Temperature	41 C
Wind Speed	3-6 mph
Soil Moisture	Dry
Soil Condition	Fine
Sun	Bright; clear skies

The soil type was a Port clay loam and due to the existing dry conditions the study was sprinkle watered with one inch of water after

alachlor was applied.

Data collected from the field study consisted of a visual rating on a scale of 0 to 10, in which 0 equaled no plant damage grading up to 10 which indicated that the plants were completely killed.

Greenhouse Study

To investigate the postemergence activity of alachlor a greenhouse study was conducted in the Oklahoma State University Weeds Laboratory, as a factorial experiment arranged in a randomized block design, with 4 replications. The day length was approximately 12 hours with a greenhouse temperature range of 16 to 32 C. Soybeans and sorghum were planted in styrofoam cups and treated when in the two true leaf stage. The commercial formulation of alachlor was applied at 0.5, 1 and 2 lb/A in 40 gallons of water per acre. Three different additives were used in combination with each rate of alachlor. They were Surfactant WK at 0.5%, a surfactant blend (Tronic) at 0.125 and 0.25% and a phytobland oil (Sun 11-E) at 0.5 and 1%.

Data collected from the postemergence greenhouse study consisted of a visual rating as to the degree of leaf burn using the 0-10 scale. Also a visual rating was taken as to the degree of malformation of the meristematic tissue and was based on 0 equaled no twisting, 1 equaled light, 2 equaled moderate and 3 equaled heavy twisting.

Growth Chamber Studies

Growth chamber studies were conducted at the Climate and Environmental Research Laboratory at Oklahoma State University to evaluate wheat, oats, sorghum (Sorghum bicolor L.) and crabgrass [Digitaria

sanguinalis (L.) Scop.] for use in biossay studies on alachlor. The studies were conducted in a randomized block design, with four replications and ten plants harvested for evaluation. The day length was 14 hours with the light source being both incandescent and fluorescent lamps at a light intensity of 2,500 ft-c. The day temperature was 30 C while the night was 24 C.

Alachlor was applied preemergence at concentrations of 0.10, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, and 3.0 parts per million (ppm) in a reciprocating nozzle spray chamber. Herbicidal response of the species was evaluated at 15 or 20 days after treatment by harvesting ten plants from each treatment, drying in an oven at 72 C and obtaining total dry weight in grams (g).

Photosynthesis (Hill Reaction Studies)

Studies of the effects of alachlor on the Hill reaction in isolated chloroplasts were conducted at the Weed Science Physiology Laboratory in the Agronomy Department at Oklahoma State University.

Chloroplasts from 14 or 21 day old wheat plants, were isolated by finely cutting up and then grinding 5 g of fresh leaves in 30 milliliters (ml) of a medium containing 10 mM EDTA, 450 mM sucrose, 50 mM boric acid, and 30 mM citric acid adjusted to pH 7.2 with NaOH. The extract was filtered through cheesecloth then centrifuged at 122 x g (1,000 rpm) for 5 minutes and the precipitate was discarded. The chloroplasts were collected by centrifugation at 4,430 x g (6,000 rpm) for 25 minutes. The chloroplasts were resuspended in 10 ml of the original buffer solution and 0.2 ml of the chloroplast suspension was added to 1.0 ml 50 mM KCl, 0.2 ml 2,6-dichloro-phenolindophenol (145 mg/liter)

with buffer to make 6.0 ml total. Where alachlor was added it replaced 1 ml of the buffer at the desired herbicide concentration. Technical alachlor was used in all of the studies. The change in optical density at 590 mμ was measured with a Klett-Summerson photoelectric colorimeter after illumination for 30-second intervals for a total of 3 minutes with a 150-watt outdoor-type spot lamp at 18 cm from the tube containing the chloroplast. The tube of chloroplast was placed in a filtering flask with tap water running through it to keep the chloroplast at a constant temperature of 24 C.

Determination of changes in optical density in the first study was made from duplications of a single chloroplast extraction. In the remaining studies the determination of changes in optical density was made in duplicates with chloroplast obtained from two different extractions. Data is presented as the arithmetical averages of the individual determinations.

Nitrate Reductase and Associated Tests

Cultural Techniques

The nitrate reductase enzyme and associated studies were conducted in a growth chamber as a factorial arrangement in a randomized block design with four replications. Duncan's multiple range statistical test was conducted at the 1% level and is indicated by small letters in the data. Technical alachlor was used in all of the studies. A day temperature of 30 C and a night temperature of 24 C was used. The day length was 14 hours and the light intensity of 2,000 ft-c with the light source being both incandescent and fluorescent lamps.

In one series of experiments, the rate of alachlor and order of

enzyme induction was studied. For these studies, wheat was cultured on perlite growth medium in 1,000 ml beakers with either a nitrogen free Hoaglands (18) nutrient solution or a Hoaglands solution containing a nitrogen source of ammonium carbonate [$(NH_3)_2$ CO_3] at a concentration of 5 mM. The plants were allowed to germinate and grow for 10 days then the solution was drained through spouts in the bottom of the beakers and replaced by a complete Hoaglands solution that contained either alachlor, nitrate [5 mM Ca $(NO_3)_2$ and 5 mM KNO_3] or some combination of alachlor and nitrate for an induction period of 17 hours.

In another series of experiments the rate of alachlor, order of enxyme induction and exposure time were varied. Wheat plants were germinated in perlite for 4 days then transferred to jars containing a nitrogen free Hoaglands solution with aeration and cultured for 6 days. Then either alachlor, nitrate or a combination of the two were introduced for a set period of time during an induction period of 15 or 16 hours.

Extraction Procedure

At the termination of the induction period an enzyme preparation was obtained by using 1 g samples of leaf tissue which was homogenized in 6 ml of a buffer solution containing 25 mM K_2HPO_4, 5 mM EDTA and 10 mM cysteine. The extraction solution was filtered through cheesecloth and centrifuged at 20,850 X g (13,000 rpm) for 15 minutes at 0 C. From this solution aliquots were used to determine nitrate content, nitrate reductase activity, amino acid and protein levels.

Nitrate Content

Wooley's (42) technique was used to determine the nitrate content. This test consisted of placing 0.2 ml of the plant extraction in 0.8 ml of distilled water and then adding 9 ml of a 20% acetic acid solution containing 0.8 g of a-Naphthylamine per liter (L) and 0.2 ppm Cu^{++} as $CuSO_4$. After cooling in icy water, 0.5 g of a powder mix containing 0.2 g of zinc dust and 50 g of sulfanilic acid was added. The test tube was inverted 3 times with a 2-3 minute interval between each inversion and kept in an ice bath for 20 minutes. An aliquot of 6 ml was poured into a clean test tube, allowed to warm to room temperature and an optical density reading was taken in a Beckman DB spectophotometer at 520 mμ Standard solutions containing 0, 5, 10, 20 and 50 μg NO_3-nitrogen per tube were prepared, read and standard curves constructed.

Nitrate Reductase Activity

The assay procedure for nitrate reductase was according to the procedure of Croy and Hageman (7). A reaction mixture was prepared which contained 0.4 ml of distilled water, 0.2 ml of 100 mM KNO_3, 0.2 ml of 1.36 mM NADH and 1.0 ml of a 50 mM PO_4 buffer solution pH 7.5 plus 0.2 ml of the plant extract. The reaction mixture was incubated at 29 C for exactly 15 minutes at which time a 1 to 1 mixture of 1% sulfanilamide reagent in 3 N HCl plus 0.02% N-(1-napthyl) ethylene diamine HCl was added. This stopped the reaction and 10 minutes were allowed for color development. The mixture was centrifuged for 10 minutes at 63 x g and read at 540 mμ in a spectophotometer.

Amino Acid Levels

Amino acid levels were measured as alpha amino nitrogen using the method of Yemm and Cocking (43). The reaction mixture contained 0.1 ml of the plant extract, 0.5 ml of a citrate buffer and 1.2 ml of a KCN-methyl cellosolve-ninhydrin mixture. The citrate buffer was prepared by dissolving 20.008 g of citric acid in 200 ml of water, adding 200 ml of 1 N NaOH and diluting to 500 ml. The KCN-methyl cellosolve-ninhydrin mixture was prepared by adding 0.1628 g of KCN in water and bringing to a volume of 250 ml. Then 5 ml of this solution were diluted to 250 ml with methyl cellosolve. Ten ml of a 5% solution (w/v) of ninhydrin in methyl cellosolve were added to 50 ml of the KCN and methyl cellosolve solution. The reaction tube tops were covered with aluminum foil, heated 15 minutes in boiling water, cooled 5 minutes in running tap water, diluted with 3 ml ethyl alcohol and read at 570 mμ in a spectophotometer. Standard solutions of isoleucine containing 5, 10, 15, 20 and 25μg nitrogen per tube were prepared, read and standard curves constructed.

Protein Content

Protein content using 5% trichloroacetic acid precipitation of water soluable protein was measured by the method of Lowery et al. (29). The procedure consisted of placing 1 ml of the plant extraction in 1 ml of 10% trichloroacetic acid, vigorously agitating and allowing mixture to set for 24 hours in the cold. After 24 hours the samples were centrifuged at 63 x g, the supernatant discarded and 3 ml of 50 mM NaOH added. The solution was agitated vigorously to allow the protein pellet to go into solution and then allowed to set 30 minutes at room

temperature. From the plant extract protein solution 0.1 ml was added to 0.9 ml of water plus 5 ml of an incubation reagent and allowed to incubate 45 minutes at room temperature. The incubation reagent contained 4% $(Na)_2$ CO_3 in 0.1 N NaOH, 4% Na tartrate and 2% $CuSO_4 \cdot 5H_2O$ which was mixed just prior to assay in a proportion of 100:1:1 ml respectively. After the incubation period 0.5 ml of a phenol color reagent plus 3.5 ml of water was added, mixed immediately, allowed to stand 30 minutes at room temperature and read at 750mμ in a spectophotometer. The phenol reagent (Folin-Ciocalteau) was a 1 to 1 mixture of a commercial phenol reagent and water. Standard solutions containing 20, 40, 80 and 160 μg of blood serum albumen per tube were prepared, read and standard curves constructed.

Uptake and Translocation Studies

Autoradiography

Soybean, an alachlor resistant species, and wheat, a susceptible species, were used to study the uptake and translocation of uniformly ring labeled ^{14}C alachlor.

The experiments were conducted in a growth chamber with a day temperature of 30 C and a night temperature of 24 C. The day length was 14 hours and the light intensity was 2,000 ft-c using incandescent and fluorescent lamps. The experiments were conducted in a randomized block design with ten replications. Duncan's multiple range statistical test was conducted at the 1% level and is indicated by small letters in the data.

For each experiment wheat and soybeans were germinated for 4 days in perlite then transferred to jars containing 300 ml of an aerated

complete Hoaglands nutrient solution for 6 days. In the root uptake study 109.8 µg of radioactive alachlor (specific activity 1.02 mc/mM, ring labled with ^{14}C) was added to 300 ml of a fresh Hoaglands nutrient solution. The plants were exposed to the ^{14}C-alachlor for a treatment period of 48 hours then removed and the roots washed for one minute under running water. The plants were then placed between 2 sheets of hardware cloth (7" by 9½") stapled on each side and vacuum freeze-dried.

In the foliar absorption study 65.68 µg of ^{14}C-alachlor plus a non-ionic surfactant at 0.5% by volume was applied to primary leaf tissue of both wheat and soybean plants in 10 one µl drops. After a 48 hour exposure period to alachlor the plants were placed between two sheets of hardware cloth and vacuum freeze-dried.

In the root uptake study the depletion of ^{14}C-alachlor from the nutrient solution at 4, 16, 28, 40 and 48 hours was determined. In the foliar uptake study the exudation of ^{14}C-alachlor into the nutrient solution was observed at 12, 24, 36, and 48 hours.

Plants treated through both the roots and foliar tissue after drying were placed in a small chamber at 100% relative humidity for 4 hours to reduce the brittleness of the tissue. The plants were then removed from the hardware cloth, mounted with Elmer's white glue on 7 by 9 inch glossy white cardboard, and pressed for 24 hours between blotters in a screw down plant press to make the plant surface as smooth as possible. The mounted plants were covered with Saran wrap and held to the cardboard with Scotch tape on the back side. In a dark room the mounted plants were placed next to the film in a Kodak Ready Pack of No Screen X-ray film. The film pack was sealed with masking tape, placed between two pieces of plywood covered with aluminum foil. The process was

repeated forming a stack of film packs, sponge rubber and plywood. The stack was fastened tightly together with two cotton web belts. An X-ray film exposure time of 30 days was used, after which the film was developed.

Liquid Scintillation Counting

After the autoradiographs were obtained the plants were sectioned into various component parts, weighed and ground in 95% ethanol with a hand, glass homogenizer. The wheat parts were homogenized in 5 ml of ethanol while the soybeans were homogenized in 10 ml of ethanol. An aliquot of 1.0 or 0.5 ml was placed in 15 ml of a counting solution depending on the degree of chlorophyll in the sample. The counting solution used was made of 5 parts xylene, 5 parts p-dioxane and 3 parts ethanol in which 80 g per liter of naphthalene was dissolved plus 5 g per liter of PPO.

The samples were counted in a liquid scintillation counter for 20 minutes or 5,000 counts whichever occurred first.

Plant Tissue Uptake

The uptake of ^{14}C-alachlor by 1 cm wheat root, coleoptile and leaf tissue was determined. The root and coleoptile tissue was 3 days old while the leaf tissue was 5 days old. The experiments were conducted in a growth chamber under continuous darkness or light with an intensity of 2,000 ft-c. A constant temperature of 30 C was maintained. The studies were conducted as a factorial arrangement in a randomized block design with ten replications.

The plant tissue was placed in 10 ml of a complete Hoaglands

nutrient solution that contained 15.3 µg of ^{14}C-alachlor. Plant tissue samples were exposed for intervals of 2, 4, 8, 16 and 32 hours after which they were placed in 15 ml of a liquid scintillation counting solution and counted.

CHAPTER IV

RESULTS AND DISCUSSION

Primary Screening Studies

Field Study

The results of a field study to evaluate the preemergence properties of alachlor on several crop and weed species are presented in Table I.

The plant species are arranged in order of the most resistant to the most susceptible of the eleven species studies. The threshold of soybean susceptibility to alachlor is greater than 8 lb/A. Cotton and morningglory (Ipomoea lacunosa L.) show resistance at levels greater than the recommended field rates for Oklahoma soils. Brachiaria [Brachiaria platyphylla (Griseb.) Nash.], Texas panicum (Panicum texanum Buckl.) and prickly sida (Sida spinosa L.) are susceptible to alachlor between 1 and 2 lb/A. The susceptibility of these three species appears to be a precise level of alachlor with very little graduation in susceptibility. Wheat, oats, and foxtail millet [Setaria italica (L.) Beauv.] were moderately susceptible while crabgrass and spiny pigweed (Amaranthus spinosus L.) were highly susceptible to alachlor.

Greenhouse Study

Limited postemergence activity of alachlor was found. Alachlor

plus phytobland oil, surfactant or surfactant blend resulted in slight leaf burn of soybean plants (Table II). Malformation of soybean meristematic tissue is shown in Table III. The addition of an additive to alachlor did not increase its herbicidal activity. Sorghum was unaffected by postemergence alachlor applications.

TABLE I

VISUAL RATING (0-10 SCALE) OF THE SUSCEPTIBILITY OF ELEVEN SPECIES TO ALACHLOR APPLIED PREEMERGENCE

Species	Alachlor Rates (lb/A)							
	0	0.25	0.50	0.75	1	2	4	8
Soybean	0	0	0	0	0	0	0	0
Cotton	0	0	0	0	0	0	2	3
Morningglory	0	0	0	0	0	0	2	6
Brachiaria	0	0	0	0	0	6	9	10
Texas panicum	0	0	0	0	0	7	9	10
prickly sida	0	0	0	0	0	9	10	10
Wheat	0	0	0	1	4	8	9	10
Oats	0	0	0	2	5	10	10	10
Foxtail millet	0	0	1	4	4	9	10	10
Crabgrass	0	1	7	9	10	10	10	10
Spiny pigweed	0	3	7	9	10	10	10	10

TABLE II

VISUAL RATING OF LEAF BURNS ON SOYBEANS RESULTING FROM ALACHLOR PLUS ADDITIVE APPLICATION

Additive	Rate[1]	Alachlor Rates (lb/A) 0	0.5	1	2
Phytobland Oil	0.50	0	0	0	1
	1.00	0	0	0	1
Surfactant Blend	0.12	0	0	0	1
	0.25	0	0	0	1
Surfactant	0.50	1	1	1	2
Check	-	0	0	1	1

[1]Percentage of carrier volume.

The application of alachlor at 1 or 2 lb/A was sufficient to cause a moderate twisting of the meristematic tissue. The twisting resembled tissue malformation resulting from an auxin type herbicide. The addition of an additive did not change the degree of malformation except where surfactant blend was added at the low rate and resulted in heavy twisting of the meristematic tissue (Table III).

Growth Chamber Studies

The utilization of oats and wheat as bioassay species for an assay period of 20 days is plotted in Figure 2. The wheat shows less deviation from a straight line response than the oats. The wheat shows greater susceptibility with increased alachlor concentration.

TABLE III

VISUAL RATING OF MERISTEMATIC TISSUE MALFORMATION RESULTING FROM ALACHLOR PLUS ADDITIVE APPLICATIONS

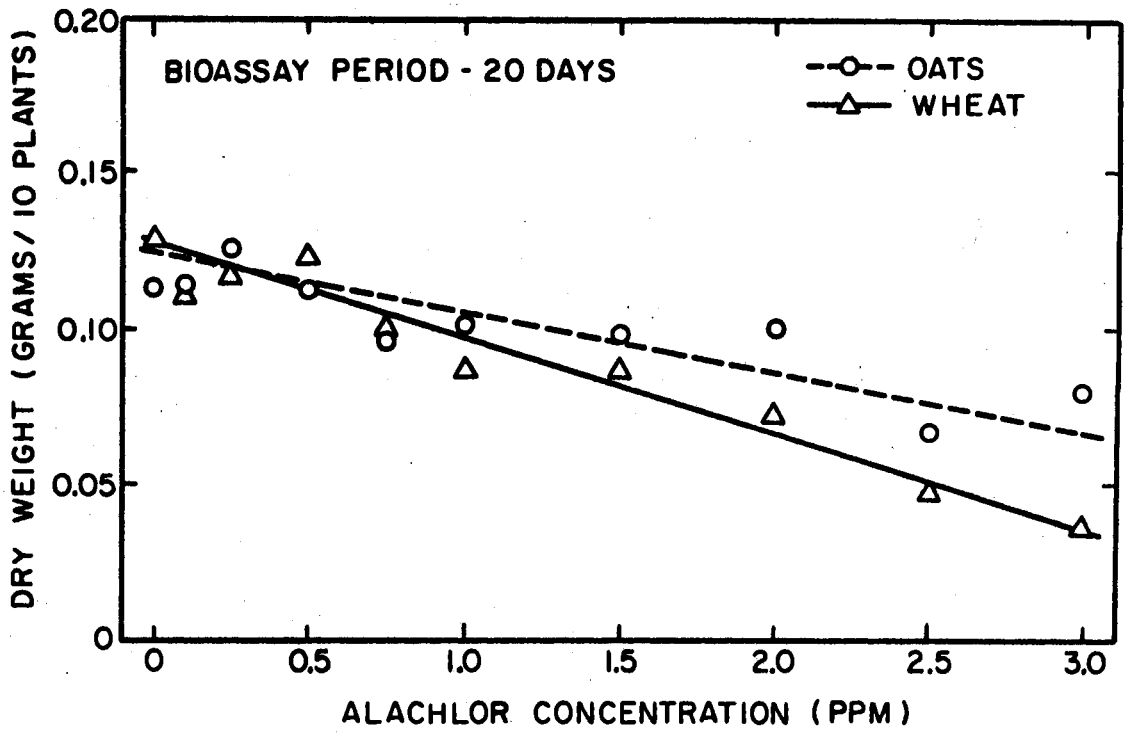
Additive	Rate[1]	Alachlor Rates (lb/A) 0	0.5	1	2
Phytobland Oil	0.50	0	0	1	2
	1.00	0	1	1	2
Surfactant Blend	0.12	0	1	1	3
	0.25	0	1	1	2
Surfactant	0.50	0	1	1	2
Check	-	0	1	2	2

[1]Percentage of carrier volume.

After considering the 20 day biossay data in Figure 2, it was decided that a shorter bioassay period might provide a better assay. Figure 2 also gives the results of a second study in which wheat, sorghum, and crabgrass were grown for a bioassay period of 15 days.

Due to the very small amount of plant material obtained it was concluded that crabgrass would not be a good bioassay species over a wide concentration range of alachlor but would be an excellent indicator of alachlor at concentrations less than 1 ppm.

It was decided from the above to use wheat as a moderately susceptible species and soybeans as a resistant species to alachlor in future studies.



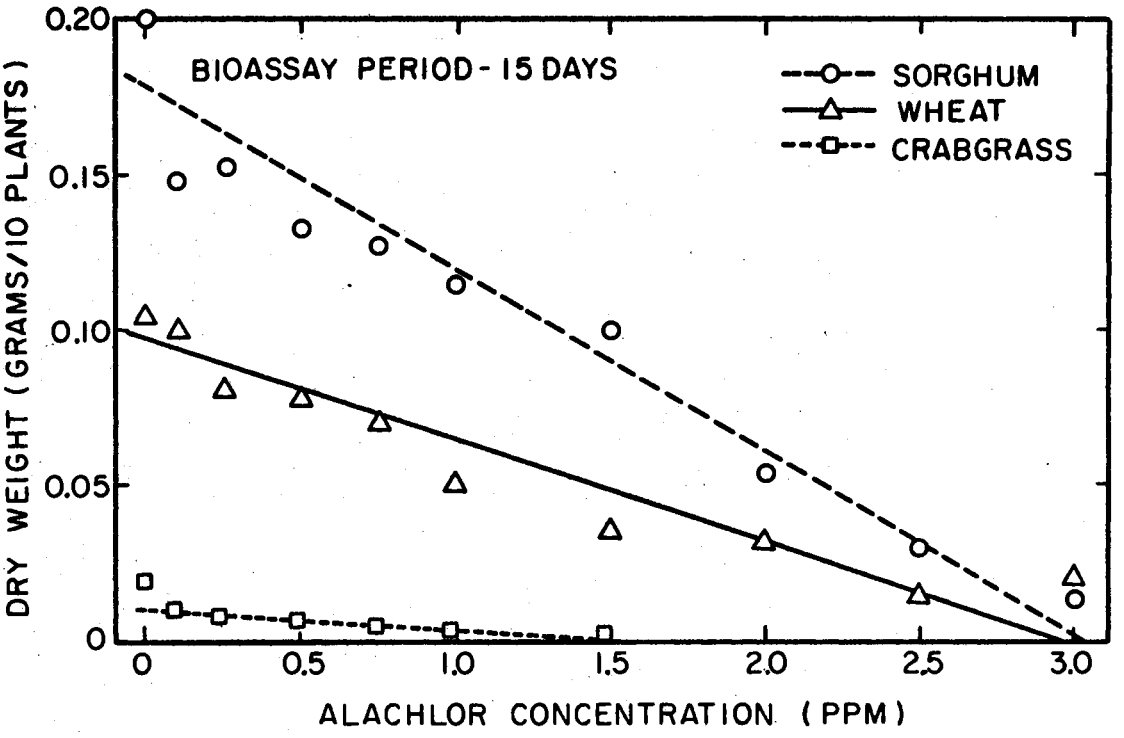


Figure 2. Biossay Response of Oats, Wheat, Sorghum and Crabgrass to Varying Alachlor Concentrations

Photosynthesis (Hill Reaction Studies)

Inhibition of the Hill reaction in isolated wheat chloroplasts from 14 day old Kaw wheat plants, did not occur with the addition of alachlor at concentrations of 5×10^{-4}, 5×10^{-5}, or 5×10^{-6} M. The difference in Klett units from time zero has been tabulated and plotted against time in Figure 3.

Since no inhibition was observed a second study was conducted with diuron at 5×10^{-4} M as a standard and alachlor at 5×10^{-4}M. Figure 4 contains the graph of the difference in Klett units from time zero as plotted against time. As the data indicates, alachlor at 5×10^{-4} M did not inhibit the Hill reaction while diuron at 5×10^{-4} M gave almost complete inhibition.

A third study was conducted using chloroplasts from 21 day old wheat plants and alachlor at concentrations of 5×10^{-4}, 5×10^{-5}, and 5×10^{-6} M. As previous studies indicated, alachlor did not inhibit the Hill reaction at any concentration under consideration (Figure 5).

It can be concluded from the above that alachlor at concentrations of 5×10^{-4}, 5×10^{-5} and 5×10^{-6} M does not inhibit or affect the Hill reaction in isolated wheat chloroplast.

It is of interest to note that alachlor does not have a free imino hydrogen atom but is replaced by a methoxymethyl group. To date, the literature seems to indicate that a free imino hydrogen atom is involved in the inhibitory action of compounds on the Hill reaction in isolated chloroplast (13,33).

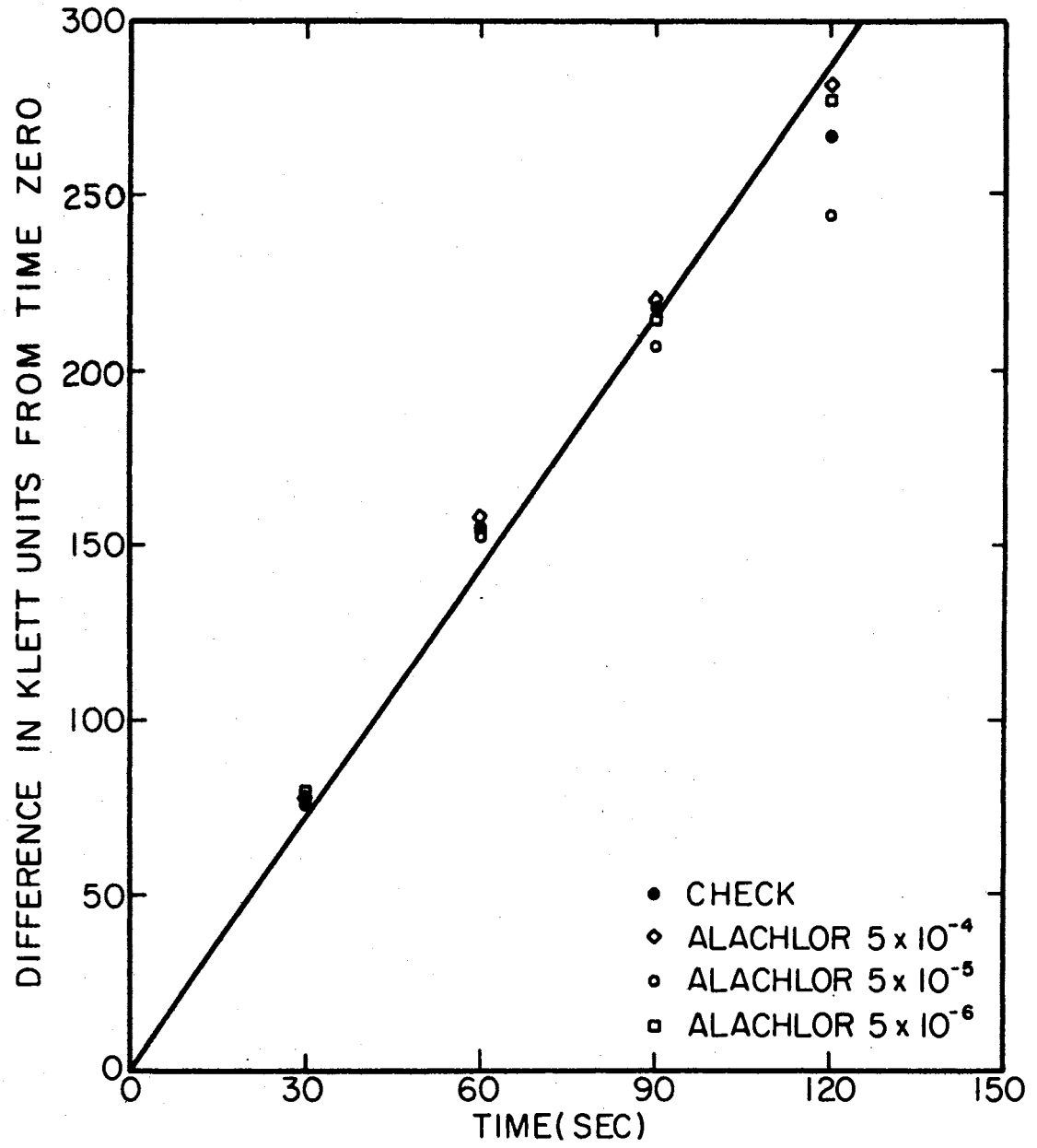


Figure 3. Effect of Alachlor on the Hill Reaction of Isolated Wheat Chloroplast (Study 1)

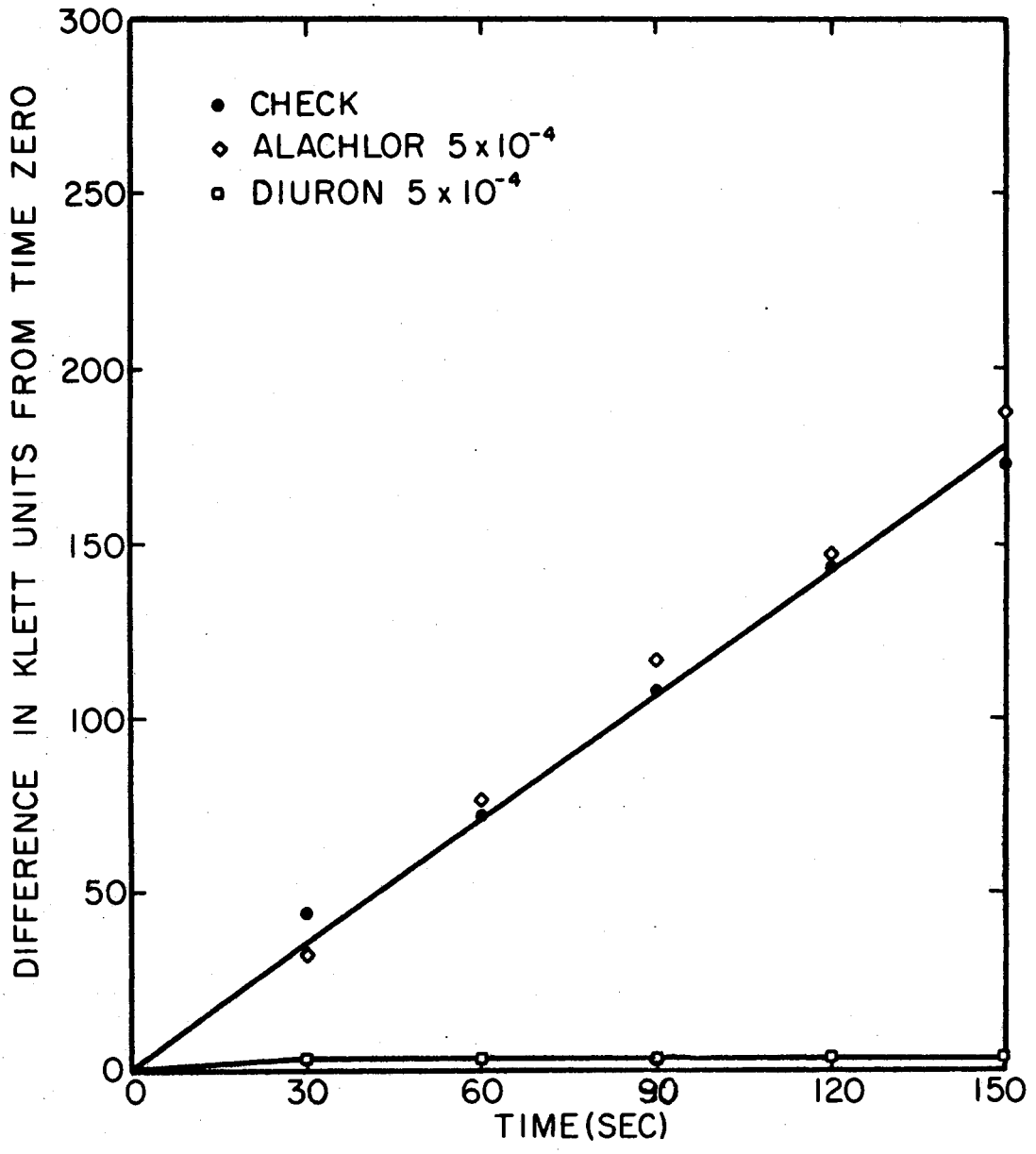


Figure 4. Effect of Alachlor and Diuron on the Hill Reaction of Isolated Wheat Chloroplast (Study 2)

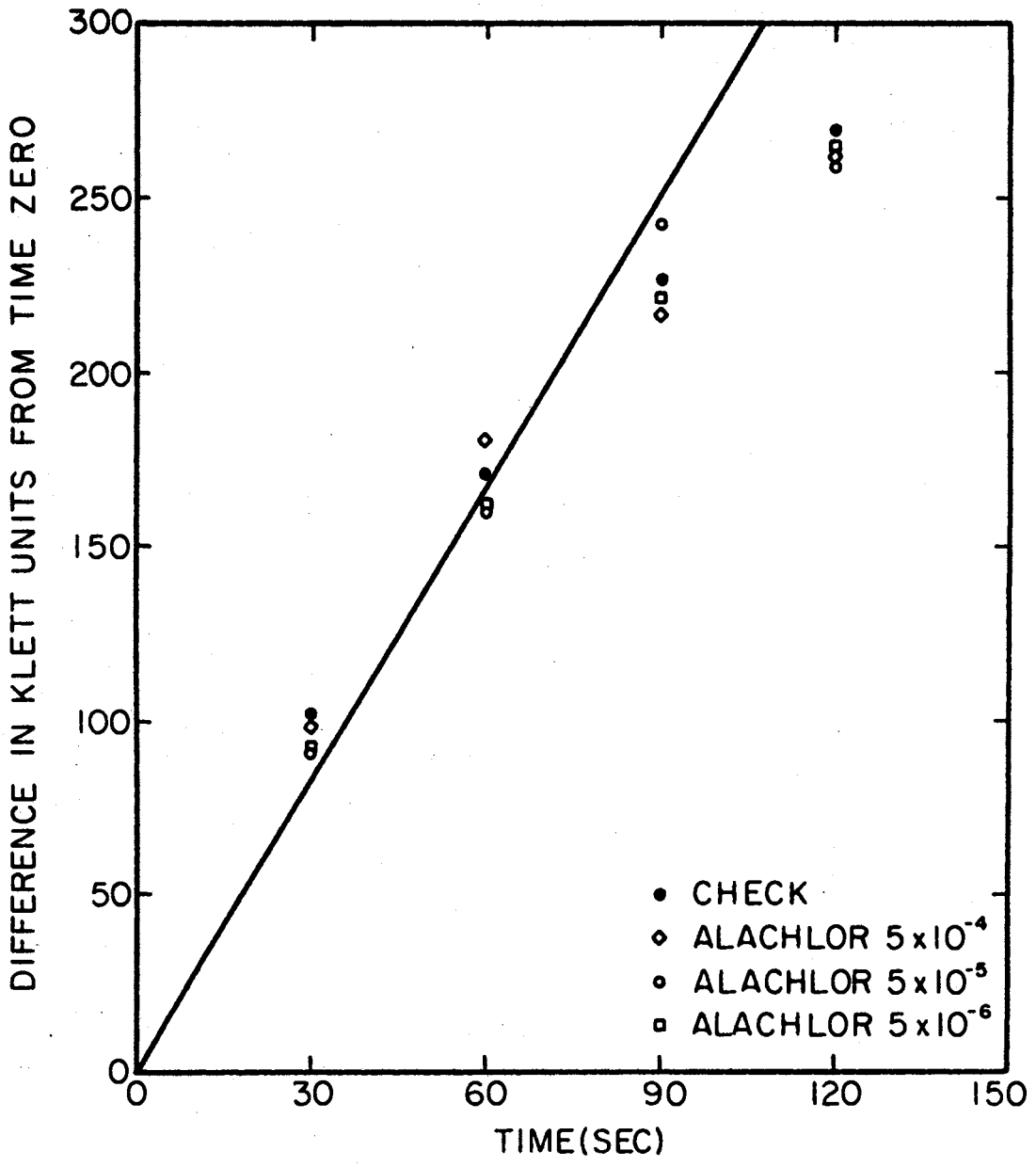


Figure 5. Effect of Alachlor on the Hill Reaction of Isolated Wheat Chloroplast (Study 3)

Nitrate Reductase and Associated Studies

Preliminary Study

A preliminary experiment showed that a low concentration of alachlor (5×10^{-6} M) did not significantly reduce the nitrate content or reduce nitrate reductase activity in wheat plants while 5×10^{-4} M did. Protein levels were not significantly affected by either level of alachlor (Table IV). All standard amino acid, nitrate and protein curves used for calculations are found in the appendix.

TABLE IV

INFLUENCE OF ALACHLOR ON NITRATE CONTENT, NITRATE REDUCTASE ACTIVITY AND PROTEIN CONTENT

Alachlor (Molar)	Nitrate[1]	Nitrate Reductase[2]	Protein[3]
0	1761 a[4]	0.147 a	38.5 a
5×10^{-6}	1660 a	0.145 a	38.0 a
5×10^{-4}	327 b	0.038 b	36.4 a

[1] $\mu g\ NO_3^N$/gm fresh weight.

[2] μ moles KNO_2/mg protein/hour.

[3] mg protein/gm fresh weight.

[4] Numbers followed by the same letter are not significantly different at the 0.01 level.

Study One

In this study wheat plants were pretreated for 7 days with either a Hoaglands solution that contained no nitrogen or one that contained nitrogen as $[(NH_3)_2\ CO_3]$. Alachlor was present throughout the 17 hour nitrate induction period. No difference was found in nitrate content or nitrate reductase activity between plants that received a pretreatment of no nitrogen or nitrogen, but were treated with alachlor (Figures 6 and 7).

The nitrate content of plants receiving a pretreatment of no nitrogen was significantly reduced by both concentrations of alachlor with the higher concentration causing a greater reduction than the lower concentration. The same response pattern resulted from both pretreatments (Figure 7). Alachlor did not significantly change the protein level at either concentration used (Table V).

Study Two

During a 17 hour nitrate induction period, alachlor was added the last 16 hours. The wheat plants were grown either with or without nitrogen for 7 days prior to treatment. Where there was no nitrogen in the pretreatment a greater nitrate content and nitrate reductase activity occurred (Figures 8 and 9).

The low concentration of alachlor caused an increase in nitrate content while the higher concentration caused almost complete blockage of nitrate uptake in plants that had received no nitrogen in the pretreatment. The nitrate content in plants pretreated with nitrogen and exposed to the high concentration of alachlor was not different from the check plants while the lower concentration caused an increase in nitrate

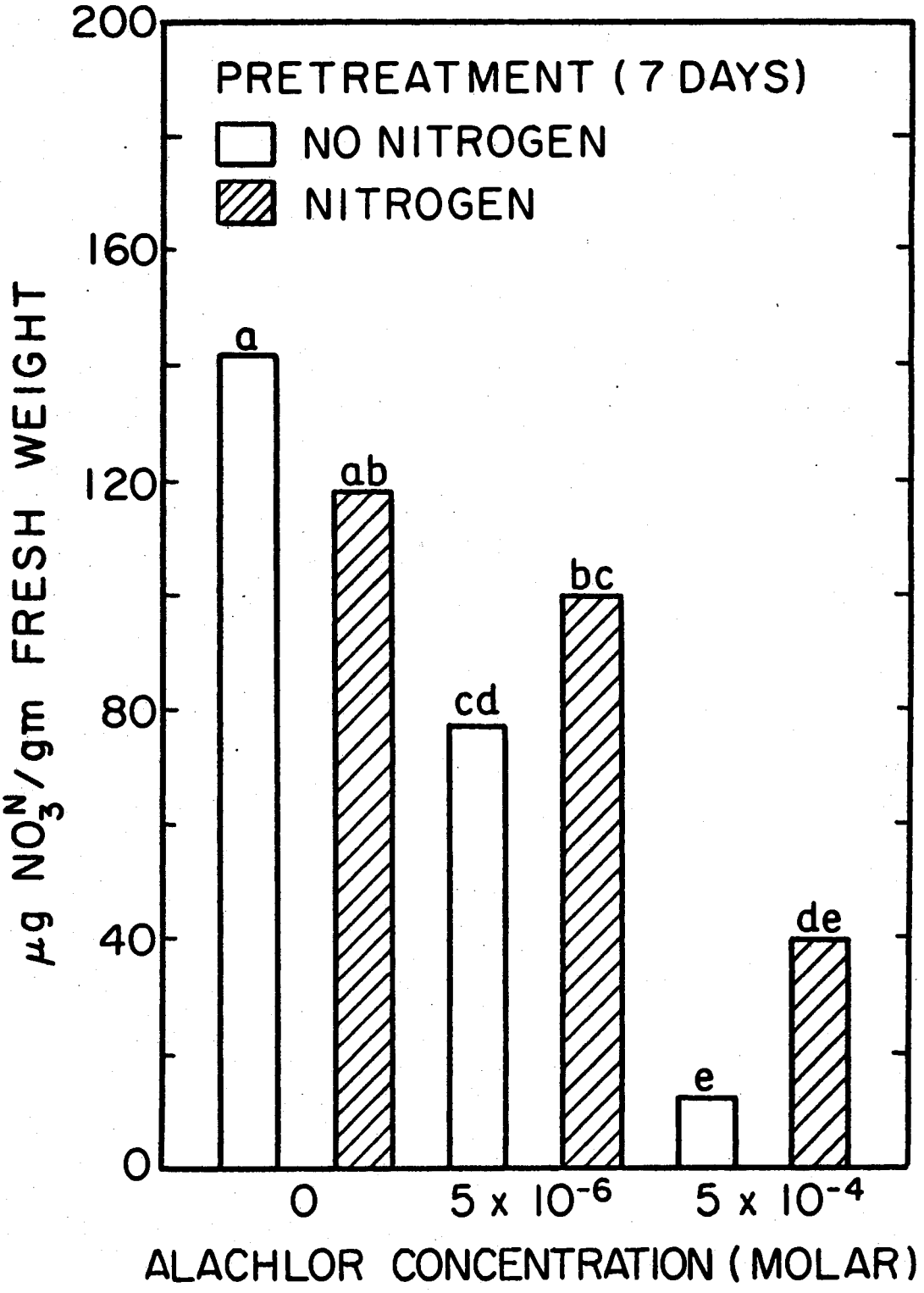


Figure 6. Influence of Alachlor on Nitrate Content: Alachlor Present Throughout 17 Hour Nitrate Induction Period

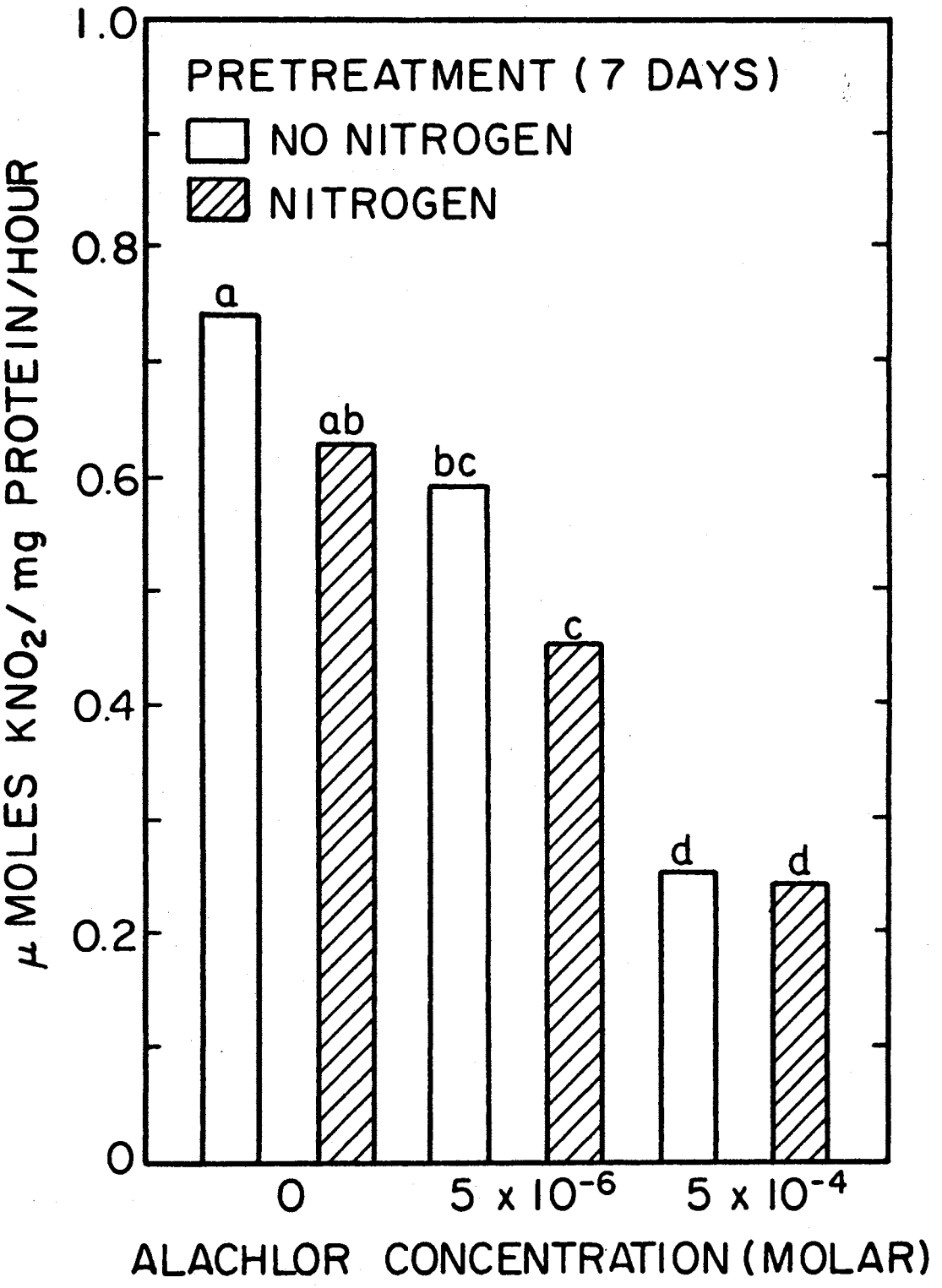


Figure 7. Influence of Alachlor on Nitrate Reductase Activity: Alachlor Present Throughout 17 Hour Nitrate Induction Period

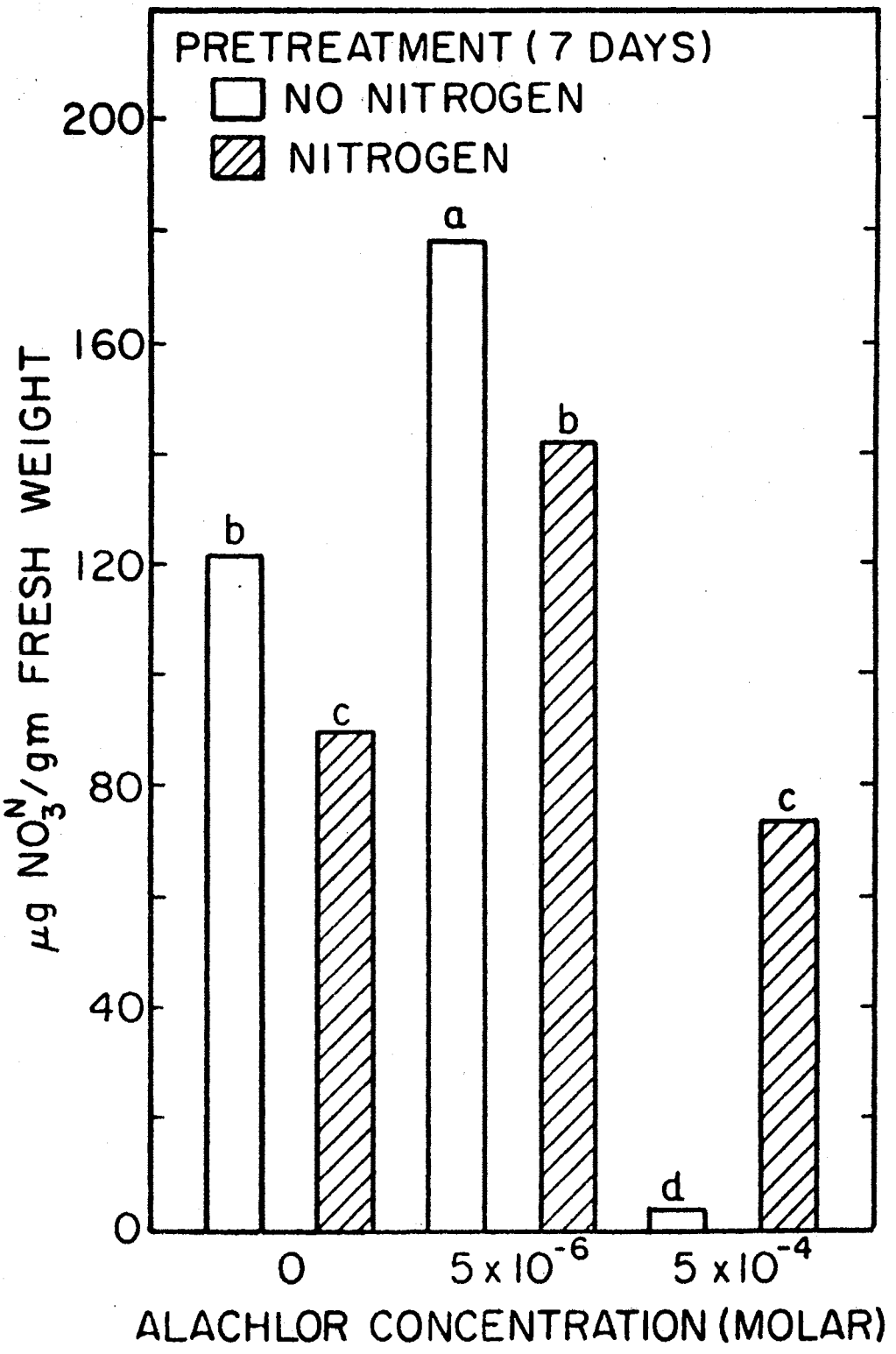


Figure 8. Influence of Alachlor on Nitrate Content: Alachlor Included the Last 16 Hours of a 17 Hour Nitrate Induction Period

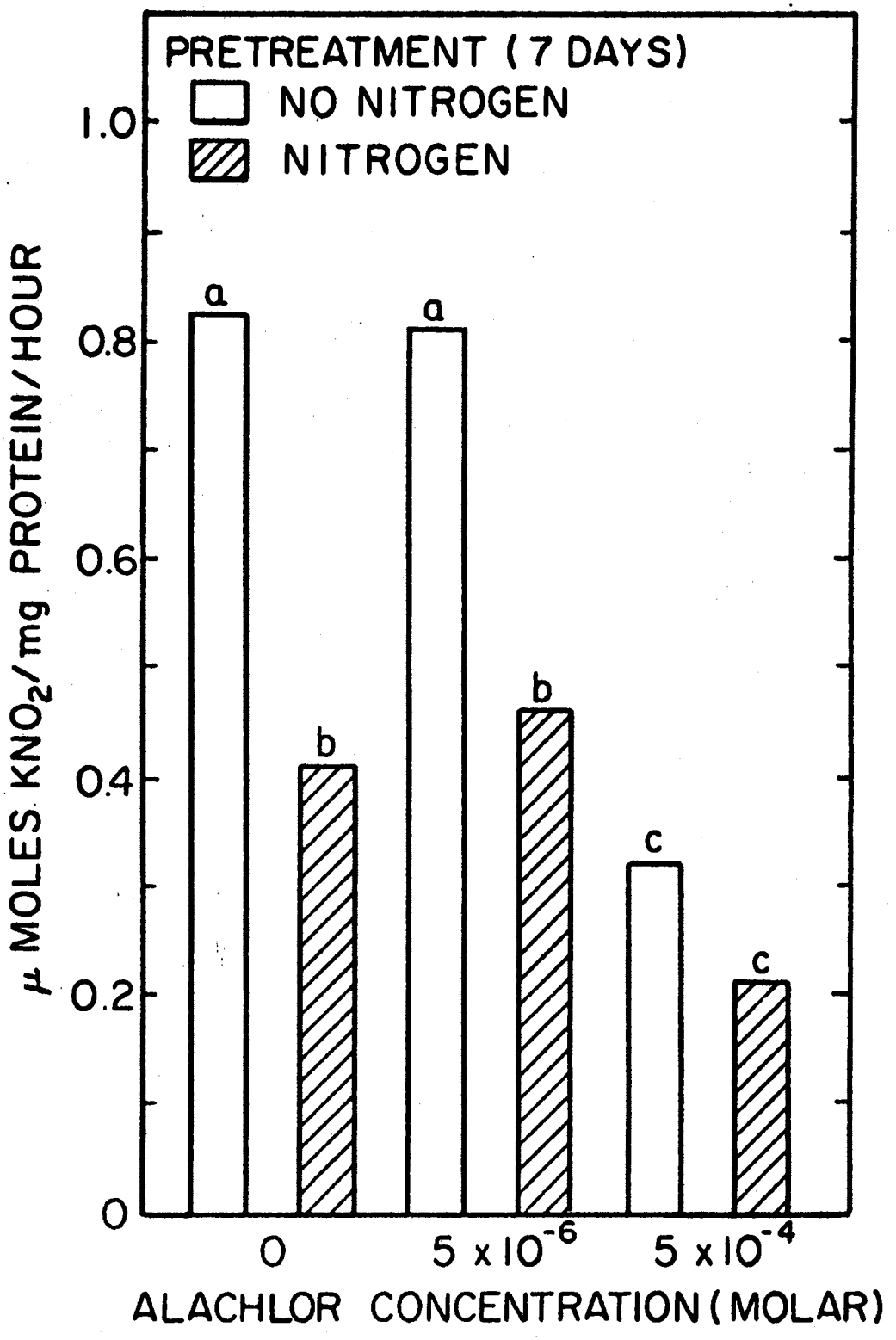


Figure 9. Influence of Alachlor on Nitrate Reductase Activity: Alachlor Included the Last 16 Hours of a 17 Hour Nitrate Induction Period

content (Figure 8).

The nitrate reductase activity was not affected by the low concentration of alachlor, but was affected by the high concentration (Figure 9). The protein content was not significantly changed by either concentration of alachlor (Table V).

Study Three

A nitrate induction period of 17 hours was used with alachlor being added for the first hour of induction. The plants pretreated with nitrogen and exposed to alachlor contained more nitrate than the plants pretreated with no nitrogen (Figure 10). The low concentration of alachlor following nitrogen pretreatment caused a stimulation in nitrate uptake while the high concentration of alachlor caused a reduction. Plants receiving no nitrogen pretreatment and exposed to the low concentration of alachlor were not different from the check while the high concentration of alachlor reduced nitrate uptake (Figure 10).

The plants receiving a nitrogen pretreatment showed less nitrate reductase activity than the plants receiving the no nitrogen pretreatment. The nitrate reductase activity of plants exposed to the low concentration of alachlor was not different from the control plants while those exposed to the high concentration showed reduced enzyme activity (Figure 11). Again, the protein content was not significantly changed by either concentration of alachlor. Plants pretreated with nitrogen had more protein than plants without nitrogen pretreatment (Table V). In Table V, it is interesting to note that the low concentration of alachlor caused a higher level of protein to exist than in the control or the high concentration of alachlor across all 3 studies.

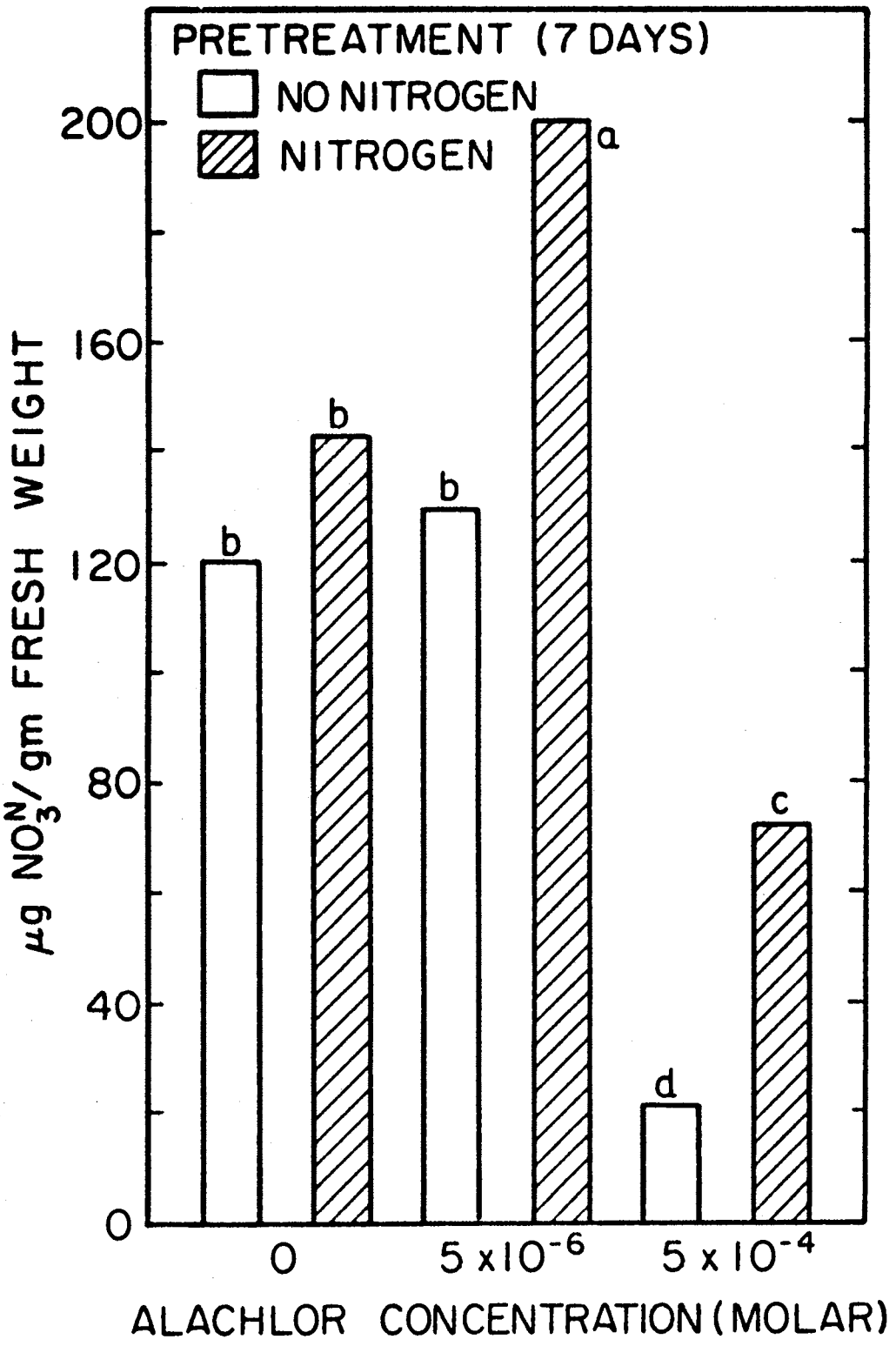


Figure 10. Influence of Alachlor on Nitrate Content: Alachlor Present During the First Hour of a 17 Hour Nitrate Induction Period

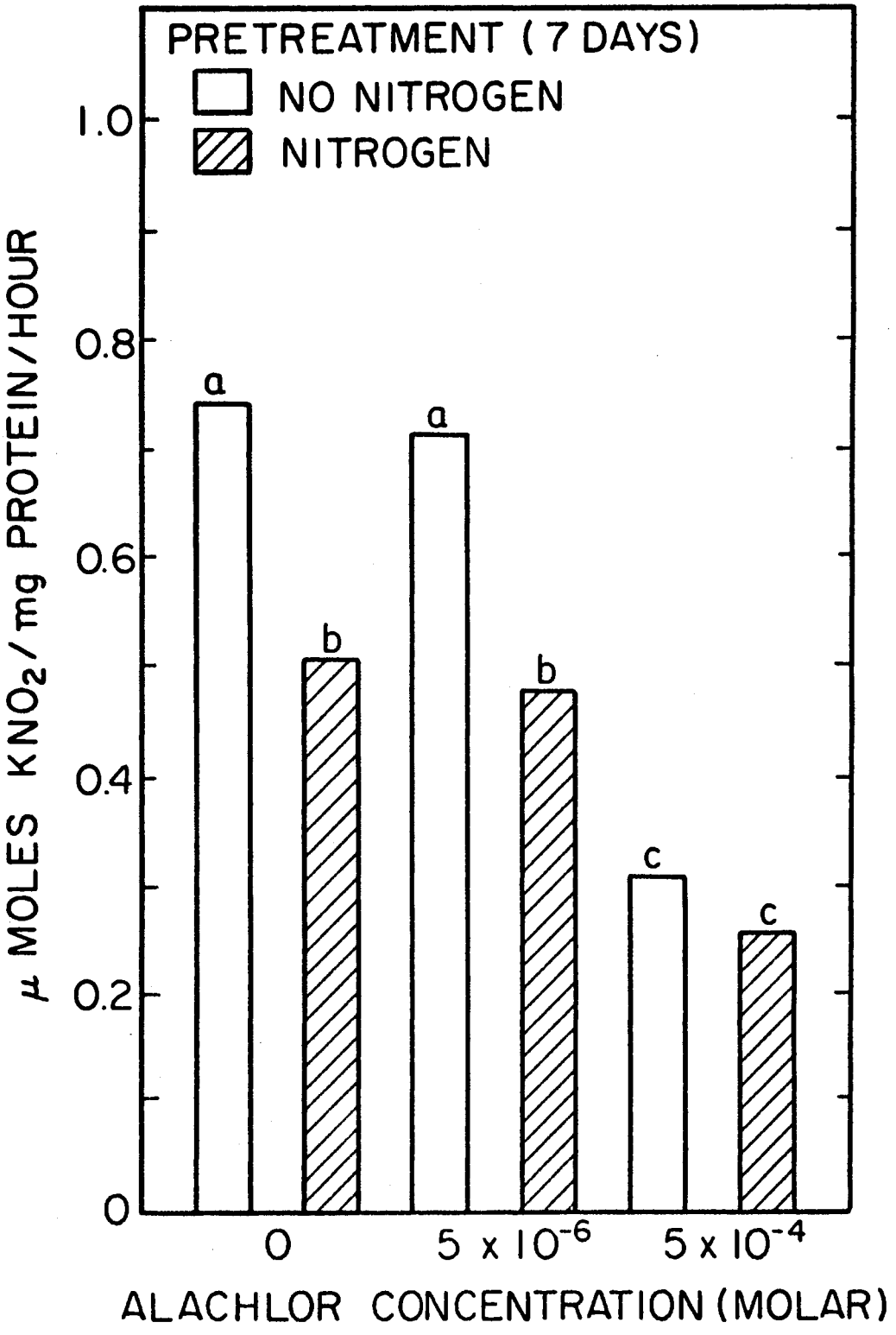


Figure 11. Influence of Alachlor on Nitrate Reductase Activity: Alachlor Present During the First Hour of a 17 Hour Nitrate Induction Period

TABLE V

INFLUENCE OF ALACHLOR ON PROTEIN CONTENT: STUDIES ONE, TWO, AND THREE

Pretreatment (7 days)	Alachlor Conc. (M)	Study Number 1	2	3
		mg protein/gm fresh weight		
No Nitrogen	0	8.61	7.94	7.13
	5×10^{-6}	8.64	7.97	7.84
	5×10^{-4}	8.05	7.94	7.10
	Blank	7.61	7.63	6.83
Nitrogen	0	9.71	9.63	9.44
	5×10^{-6}	10.40	9.72	9.82
	5×10^{-4}	9.96	9.41	8.55
	Blank	10.06	9.66	8.90

The utilization of $(NH_3)_2\ CO_3$ at a concentration of 5 mM in studies 2, 3 and 4 caused slight to moderate stunting of the wheat plant. Weissman (41) demonstrated that ammonia ions can markedly inhibit the uptake of nitrate ions by wheat seedling. Minotti et al. (31) suggested that ammonium, and to some extent the high acidity adjacent to cellular boundary membranes resulting from ammonium uptake in excess of nitrate uptake, resulted in alterations in membrane permeability, thereby restricting capacity for nitrate absorption.

The above may help explain why there was a significant reduction in nitrate content in the control of study 2 where plant tissue was pretreated with nitrogen as compared to the no nitrogen pretreatment.

Study Four

In studies 4 and 5 the wheat plants were not grown on the perlite but suspended in a nitrogen free nutrient solution. Due to the toxic effect of $(NH_3)_2\ CO_3$, it was eliminated from the pretreatment in studies 4 and 5.

In this experiment the time interval of alachlor exposure was considered. A nitrate reductase induction period of 15 hours was used with alachlor concentrations of 5×10^{-6} and 5×10^{-4}. In this study the nitrate content was reduced by both concentrations of alachlor with the higher concentration being more inhibitory. There was a reduction in nitrate content with an increase in total exposure time to alachlor during the first half (0-8 hours) of the induction period. During the second half (8-15 hours) of the induction there was little difference in nitrate content with increased exposure time (Figure 12).

As with nitrate content, both concentrations of alachlor caused a reduction in nitrate reductase activity and longer alachlor exposure times further decreased the nitrate reductase activity (Figure 13). This reduction in nitrate reductase activity may be a reflection of the reduction in nitrate ion uptake.

Addition of alachlor for 2 hours caused a slight increase in the amino acid content. If alachlor were present for more than 4 hours during the induction period, there was a slight reduction in amino acid content.

The higher concentration (5×10^{-4}) of alachlor reduced the amino acid content when averaged over time, but the low concentration did not. The reduction in amino acid content may be a reflection of the reduced nitrate reductase activity and nitrate ion uptake (Figure 14).

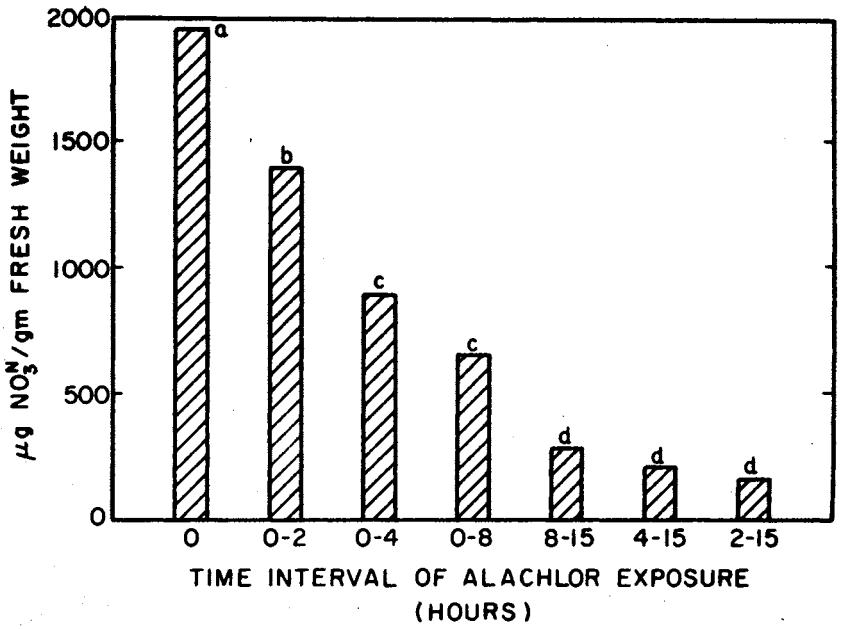


Figure 12. Nitrate Content as Affected by Exposure Time to Alachlor (Average of 5 X 10^{-4} and 5 X 10^{-6} M Concentrations) During a 15 Hour Induction Period

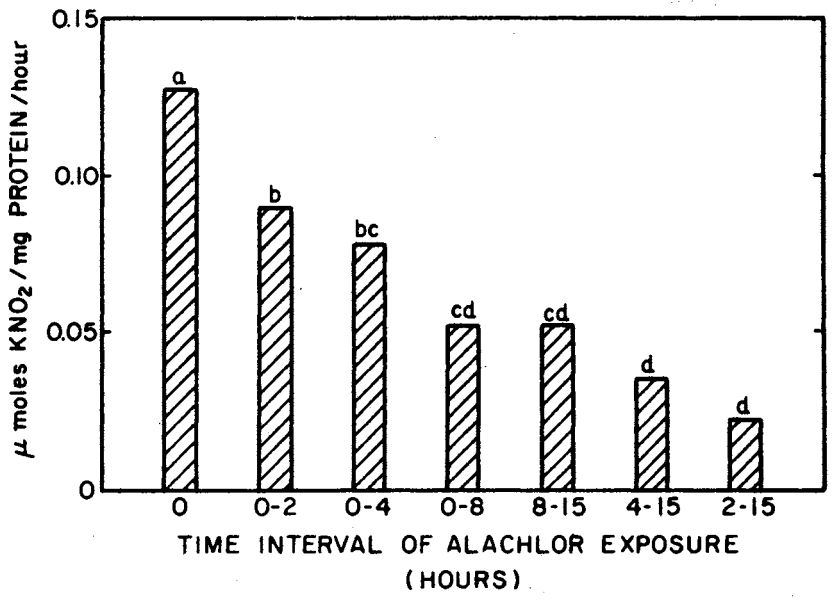


Figure 13. Nitrate Reductase Activity as Affected by Exposure Time to Alachlor (Average of 5 X 10^{-4} and 5 X 10^{-6} M Concentrations) During a 15 Hour Induction Period

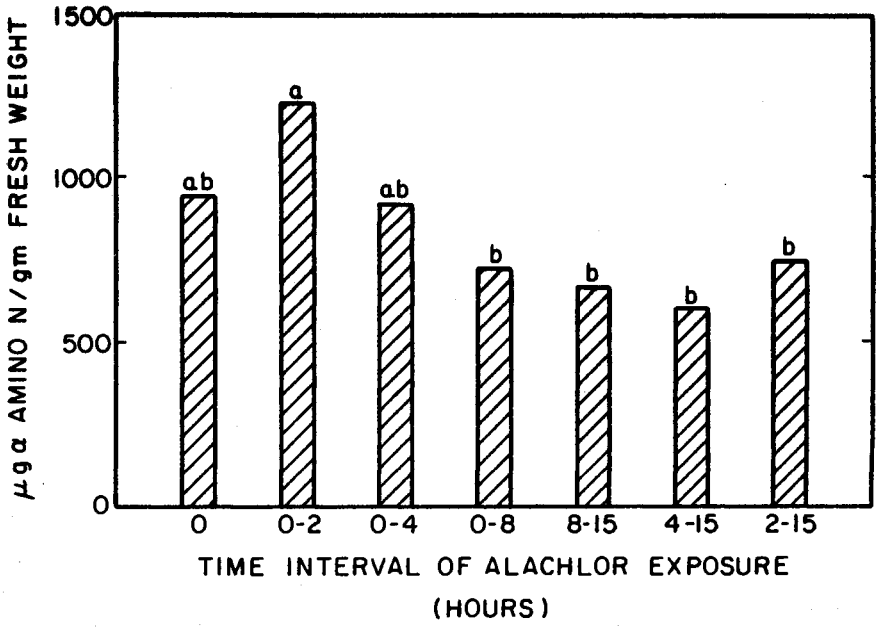


Figure 14. Amino Acid Content as Affected by Exposure Time to Alachlor (Average of 5 X 10^{-4} and 5 X 10^{-6} M Concentrations) During a 15 Hour Induction Period

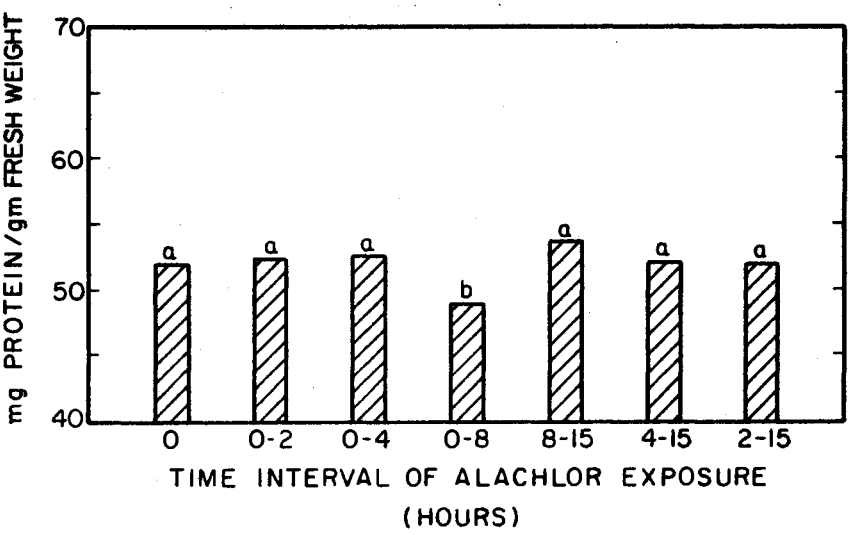


Figure 15. Protein Level as Affected by Exposure Time to Alachlor (Average of 5 X 10^{-4} and 5 X 10^{-6} M Concentrations) During a 15 Hour Induction Period

The addition of alachlor for at least 8 hours at the beginning of induction caused a significant reduction in protein content. Other time intervals of alachlor addition did not affect protein content (Figure 15).

Study Five

In this experiment the plants were exposed to alachlor for varying time periods before or after nitrate induction. Alachlor was used at concentrations of 5×10^{-6} and 5×10^{-4} for 4, 8, 12 and 16 hours, with the total reaction period being 16 hours. The asterisk in each figure indicates the level of response if no alachlor was added during induction with nitrate.

The addition of alachlor after nitrate in general caused a greater reduction in nitrate content than the addition of alachlor before nitrate (Figure 16). Exposure of alachlor for greater than 8 hours after nitrate reduced the nitrate content while 4 hours of alachlor following 12 hours of nitrate was not different from 16 hours of nitrate (Figure 17). The addition of alachlor alone for 4 hours before being replaced with nitrate resulted in a significant stimulation of nitrate content. This occurred with both concentrations of alachlor. If alachlor were added for 8 hours or longer, a reduction in nitrate content occurred (Figure 18).

The addition of alachlor before nitrate caused a significantly greater reduction in nitrate reductase activity than the addition of alachlor after nitrate (Figure 19). With twelve hours of nitrate followed by 4 hours of alachlor, there was an increase in nitrate reductase activity with both concentrations of alachlor. Eight hours of

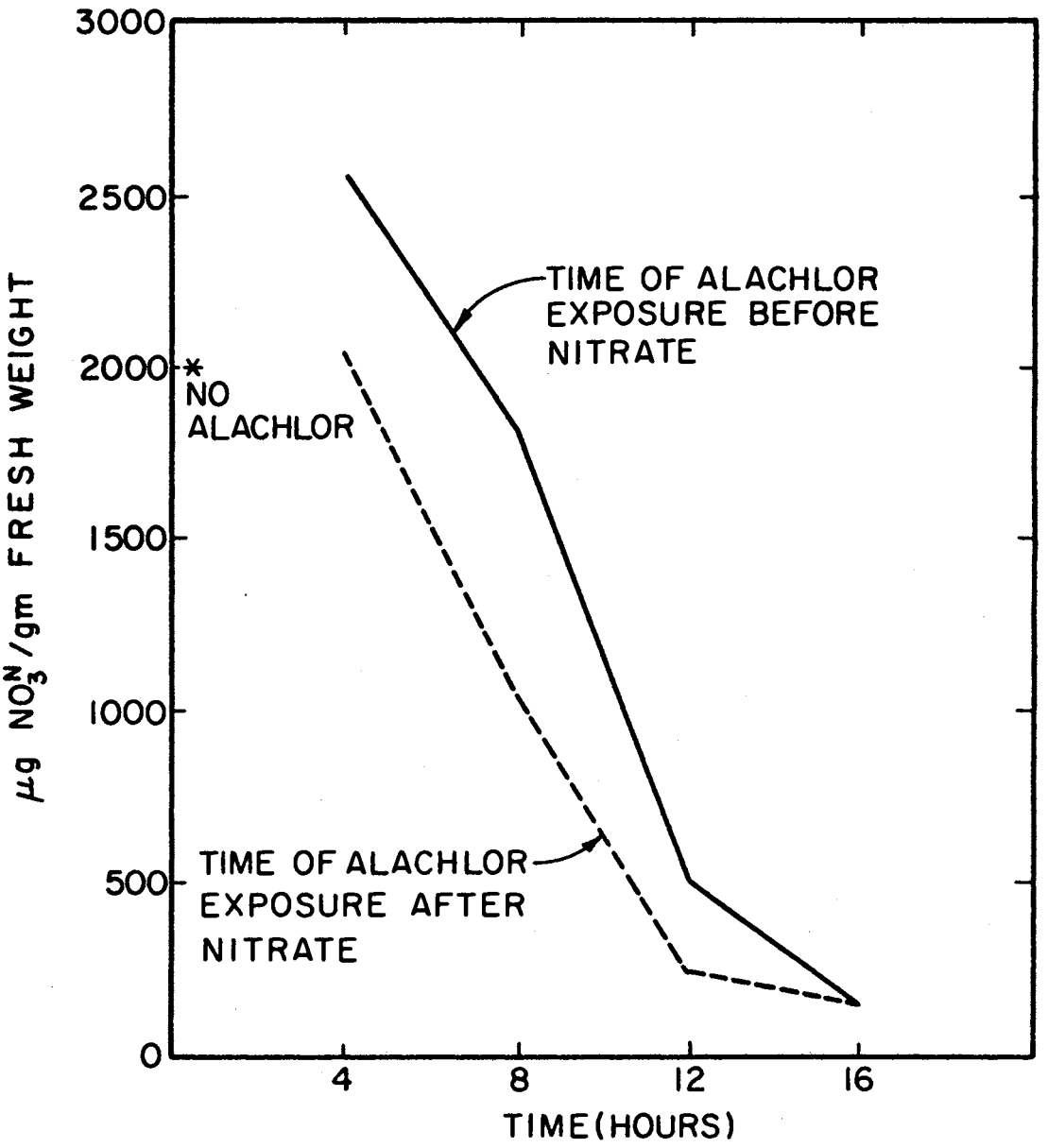


Figure 16. Influence of Alachlor on Nitrate Content: Alachlor (Average of 5 X 10^{-5} and 5 X 10^{-6} M Concentrations) Present Either Before or After Nitrate was Added

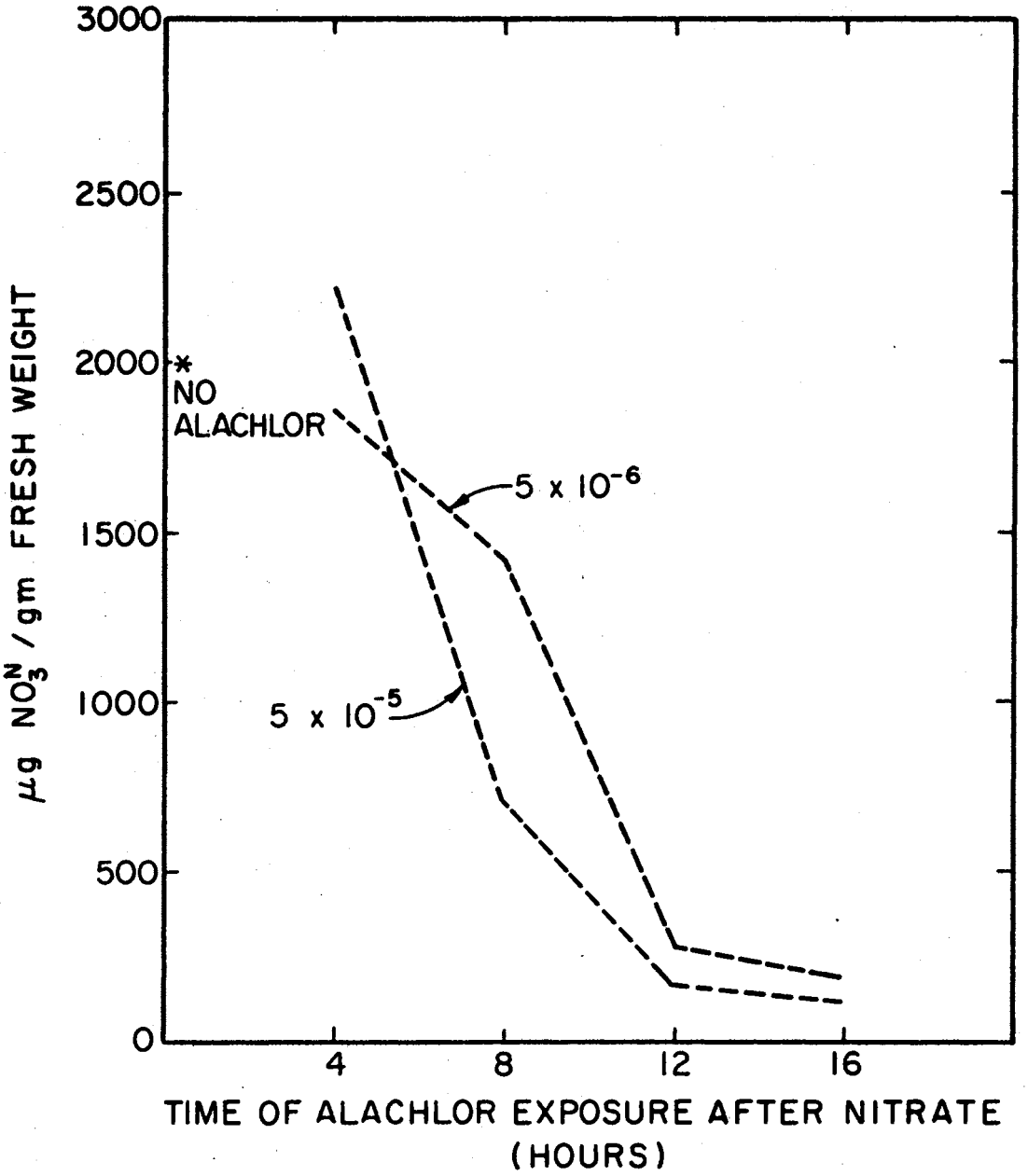


Figure 17. Influence of Alachlor on Nitrate Content: Alachlor Added After Nitrate

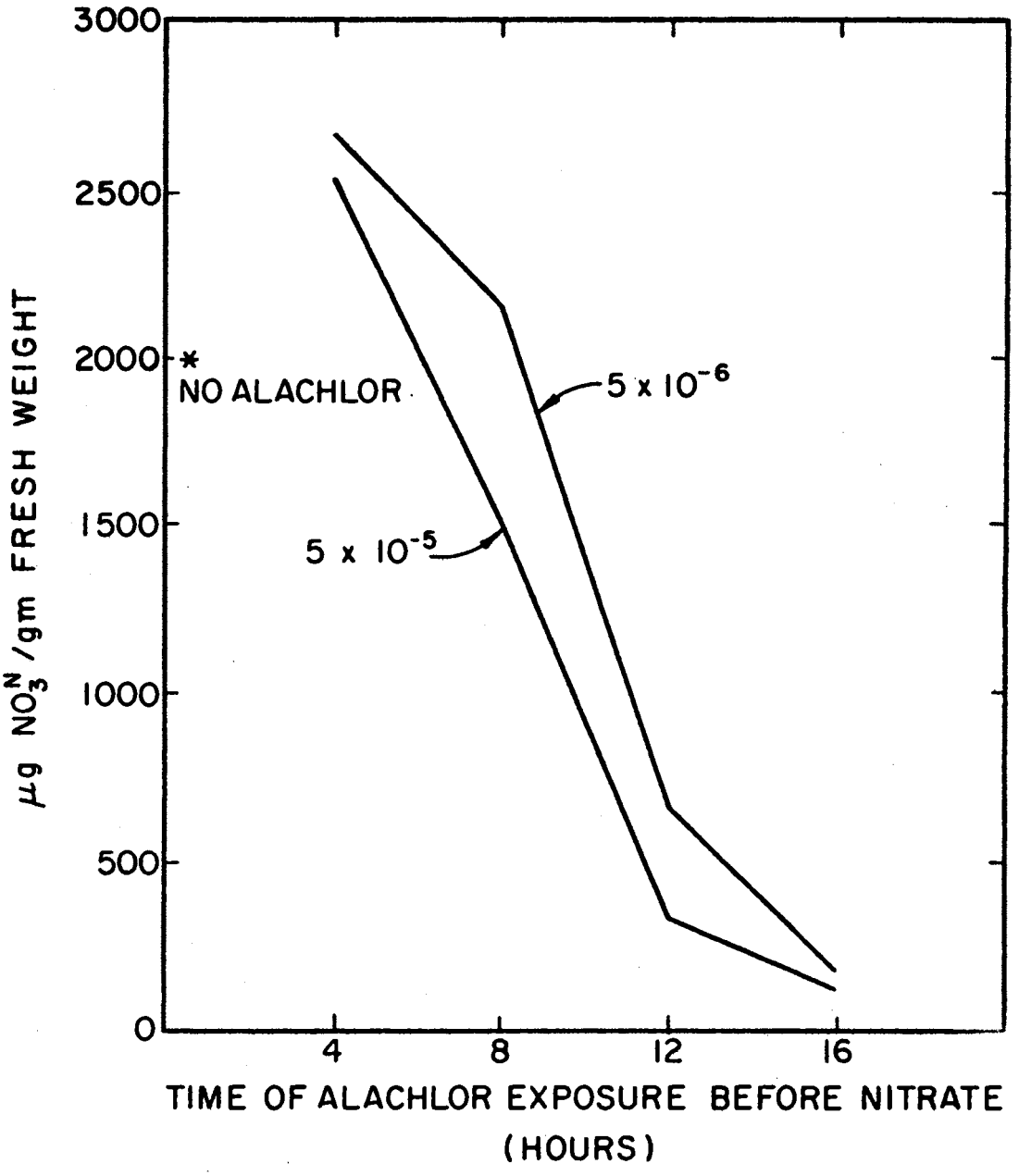


Figure 18. Influence of Alachlor on Nitrate Content: Alachlor Added Before Nitrate

nitrate followed by alachlor caused a reduction in nitrate reductase activity at the higher concentration while a stimulation occurred at the lower concentration. An alachlor exposure of greater than 8 hours following nitrate caused a reduction in enzyme activity at both concentrations of alachlor Figure 20).

When alachlor was applied first for 4 hours followed by 12 hours of nitrate, there was no difference between it and 16 hours of nitrate. At 8 hours of alachlor or greater followed by nitrate there was a significant reduction in nitrate reductase activity (Figure 21).

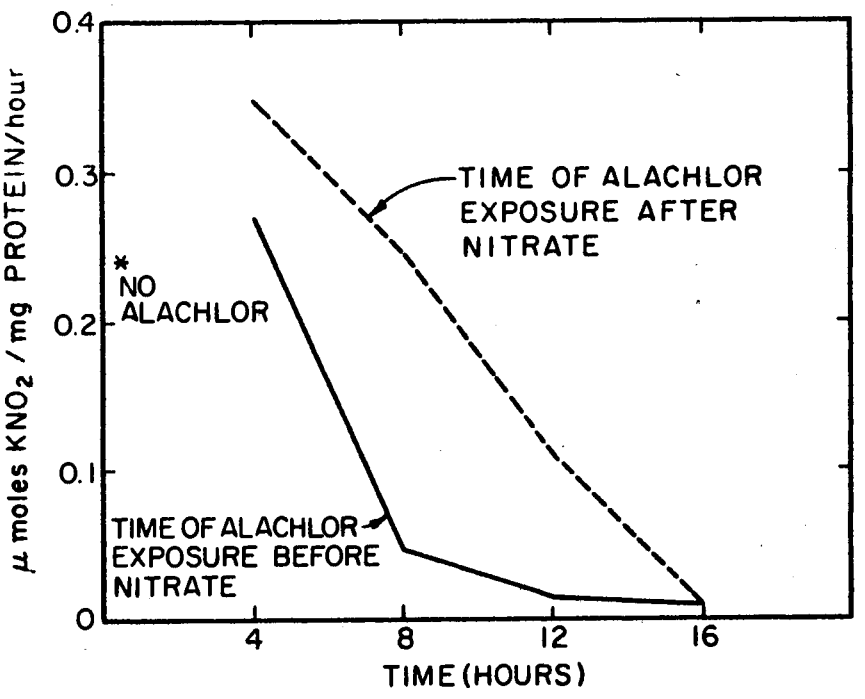


Figure 19. Influence of Alachlor on Nitrate Reductase Activity: Alachlor (Average of 5 X 10^{-5} and 5 X 10^{-6} M Concentrations) Present Either Before or After Nitrate Was Added

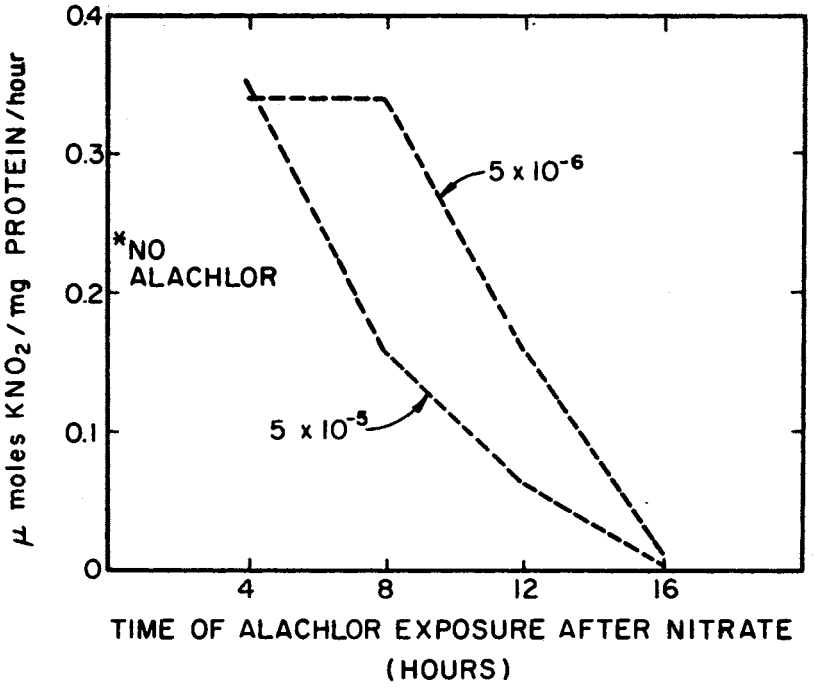


Figure 20. Influence of Alachlor on Nitrate Reductase Activity: Alachlor Added After Nitrate

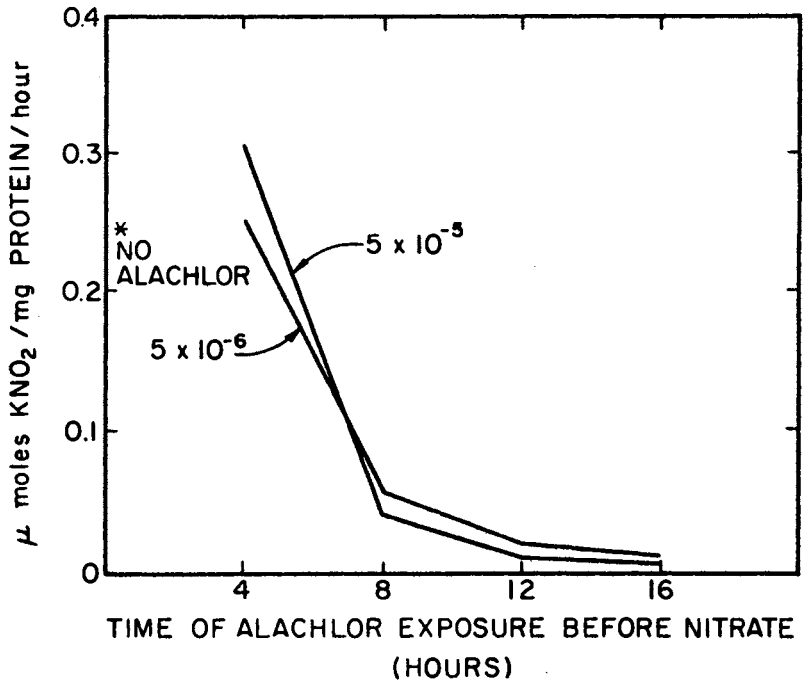


Figure 21. Influence of Alachlor on Nitrate Reductase Activity: Alachlor Added Before Nitrate

The nitrate ion concentration at 8 hours was higher in the 5×10^{-6} M alachlor treatment than in the no alachlor treatment and yet nitrate reductase activity was much lower (Figures 18 and 21). The nitrate concentration was depressed somewhat by alachlor at 5×10^{-5} M while the nitrate reductase was depressed very markedly. This would indicate that nitrate reductase was being inhibited by alachlor.

The amino acid content shows a similar trend to the nitrate content but the rate of decrease in content is not as great. Both the amino acid content and nitrate reducrase activity are depressed by alachlor but the effect seems to be greater on nitrate reductase activity than on the amino acid content. In general, as the total time exposure of alachlor is increased, the amino acid content decreased (Figures 22 and 23).

As Figure 24 shows when averaged across concentrations, the addition of alachlor before or after nitrate did not significantly affect the protein content. When averaged across time, the higher concentration of alachlor caused a significant reduction in protein while the lower concentration did not (Figure 25).

The enzyme and associated studies show that alachlor may stimulate or inhibit both nitrate ion uptake or nitrate reductase activity depending on the concentration used, order of exposure or the total time of exposure. Under certain conditions the amino acid content may be lowered depending on the exposure time to alachlor. With a high enough concentration of alachlor, the protein level may be reduced.

Since alachlor is applied preemergence, it would be worth while to consider the above data from that stand point.

A developing seedling obtains the required growth substances for

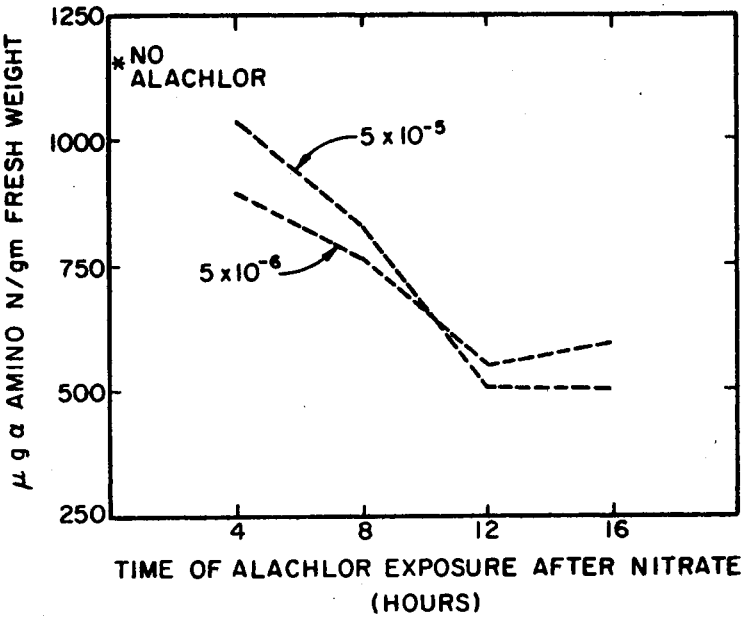


Figure 22. Influence of Alachlor on Amino Acid Content: Alachlor Added After Nitrate

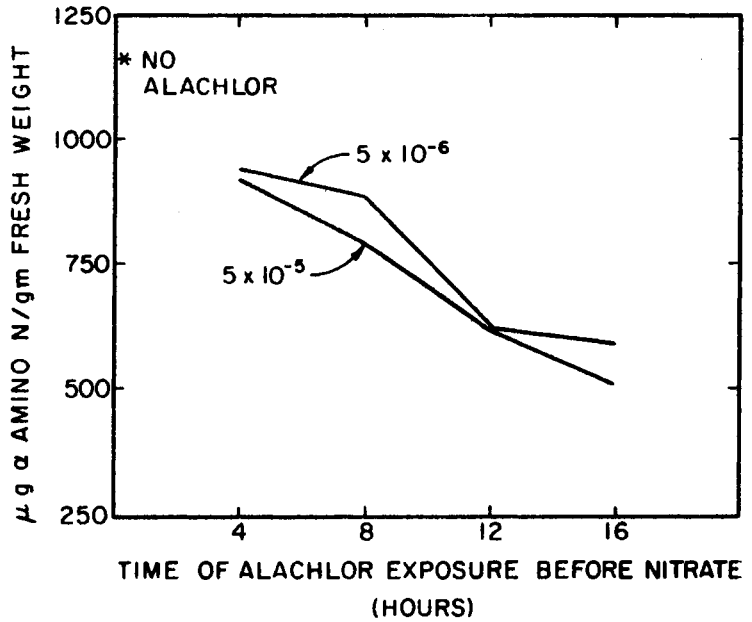


Figure 23. Influence of Alachlor on Amino Acid Content: Alachlor Added Before Nitrate

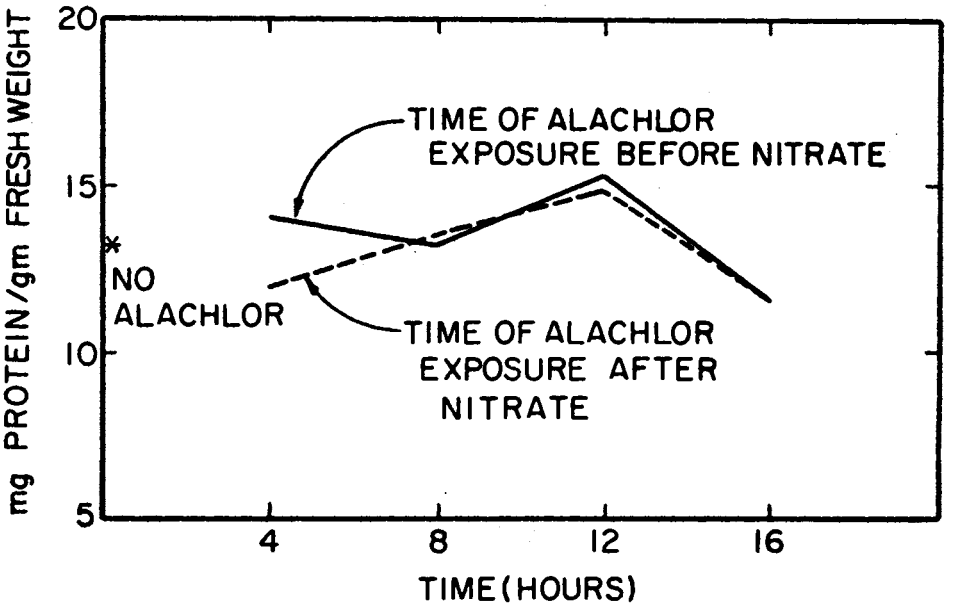


Figure 24. Influence of Alachlor on Protein Level: Alachlor (Average of 5 X 10^{-5} and 5 X 10^{-6} M Concentrations) Present Either Before or After Nitrate was Added

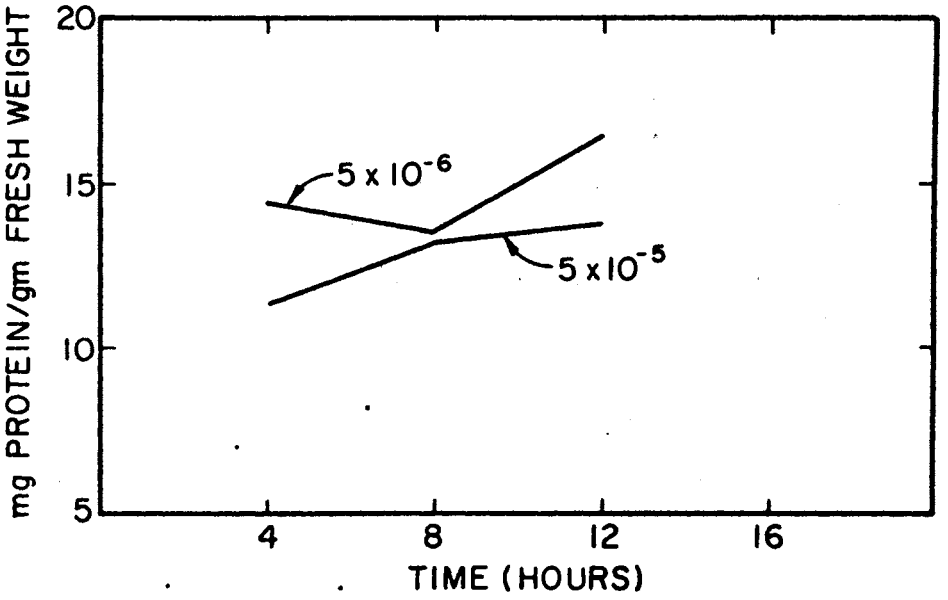


Figure 25. Influence of Alachlor Concentrations on Protein Level

the seed until sufficient root and foliage has developed to support the plant. The developing seedling will obtain nutrients from the soil media early in the germination stage. In a developing seedling the induction of nitrate reductase will be set into motion with the absorption of nitrate and light. Prior to nitrate reductase induction, adequate nitrogen for plant development is obtained from the endosperm of the seed.

About the same time the roots start absorbirg nutrients from the soil, the coleoptile is approaching or emerging through the soil surface. From wheat tissue uptake studies, it is known that the coleoptile tissue is capable of absorbing alachlor. Therefore in the development of a seedling in soil where alachlor has been applied preemergence, the uptake of nitrate and induction of nitrate reductase may occur before, after or simultaneous to the absorption of alachlor.

If uptake of nitrate and alachlor at a sufficient concentration occurred simultaneously, the nitrate content and nitrate reductase activity could be substantially reduced. If the uptake of nitrate precedes alachlor absorption, low alachlor concentrations could stimulate nitrate uptake while higher rates could be inhibitory. The nitrate reductase activity could be reduced if high enough concentrations of alachlor accumulate. If alachlor uptake occurred prior to enzyme induction, the nitrate content could be enhanced or reduced depending on the concentration of alachlor and exposure period before nitrate uptake. The enzyme activity would be reduced drastically if the time of alachlor exposure before nitrate uptake were 8 hours or greater.

The amino acid and protein levels may be reduced if sufficient

alachlor concentration and exposure time occurs.

After studying the effects of alachlor on the enzyme, nitrate reductase, nitrate content, amino acid and protein levels of wheat, it seems likely that alachlor affects several systems. Since nitrate uptake is affected, the enzyme system which is responsible for moving nitrate into the cell may also be sensitive to alachlor.

Uptake and Translocation Studies

Autoradiography

In studying the uptake and translocation of alachlor, uniformly ring labled ^{14}C-alachlor was applied to soybeans, a resistant species, and wheat, a susceptible species.

The absorption of ^{14}C-alachlor by intact wheat roots resulted in the autoradiogram shown in Figure 26. Alachlor moved uniformly throughout the entire wheat plant with greater accumulation of alachlor in the roots than in the foliar tissue.

Intact soybean root absorption of ^{14}C-alachlor resulted in the autoradiogram shown in Figure 27. As in the wheat, alachlor moved uniformly throughout the entire soybean plant when entering through the roots with greater accumulations of alachlor in the roots than in the filiar tissue.

Ten one-µl droplets of ^{14}C-alachlor were applied to the primary leaf tissue of 10 day old wheat plants. On the wheat leaf blade the ^{14}C-alachlor was placed along one side of the blade so untreated tissue could absorb and transport alachlor in a basepetal or acropetal direction without restrictions. The autoradiogram in Figure 28 shows a high concentration of alachlor at the area of application. Figure 28 also

Figure 26. Autoradiograph of Wheat Plant: ^{14}C-Alachlor Applied Through the Roots

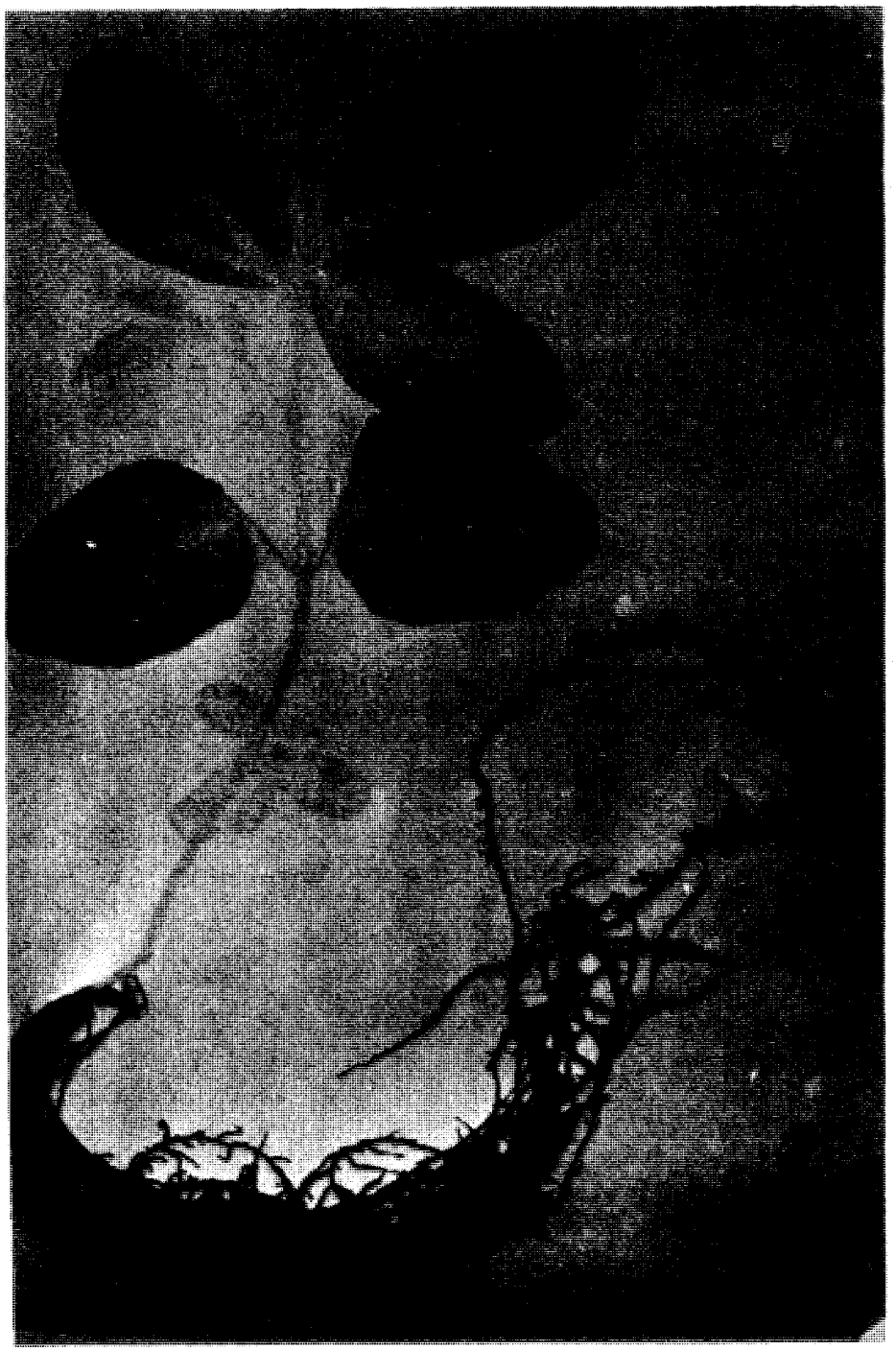


Figure 27. Autoradiograph of Soybean Plant: ^{14}C-Alachlor Applied Through the Roots

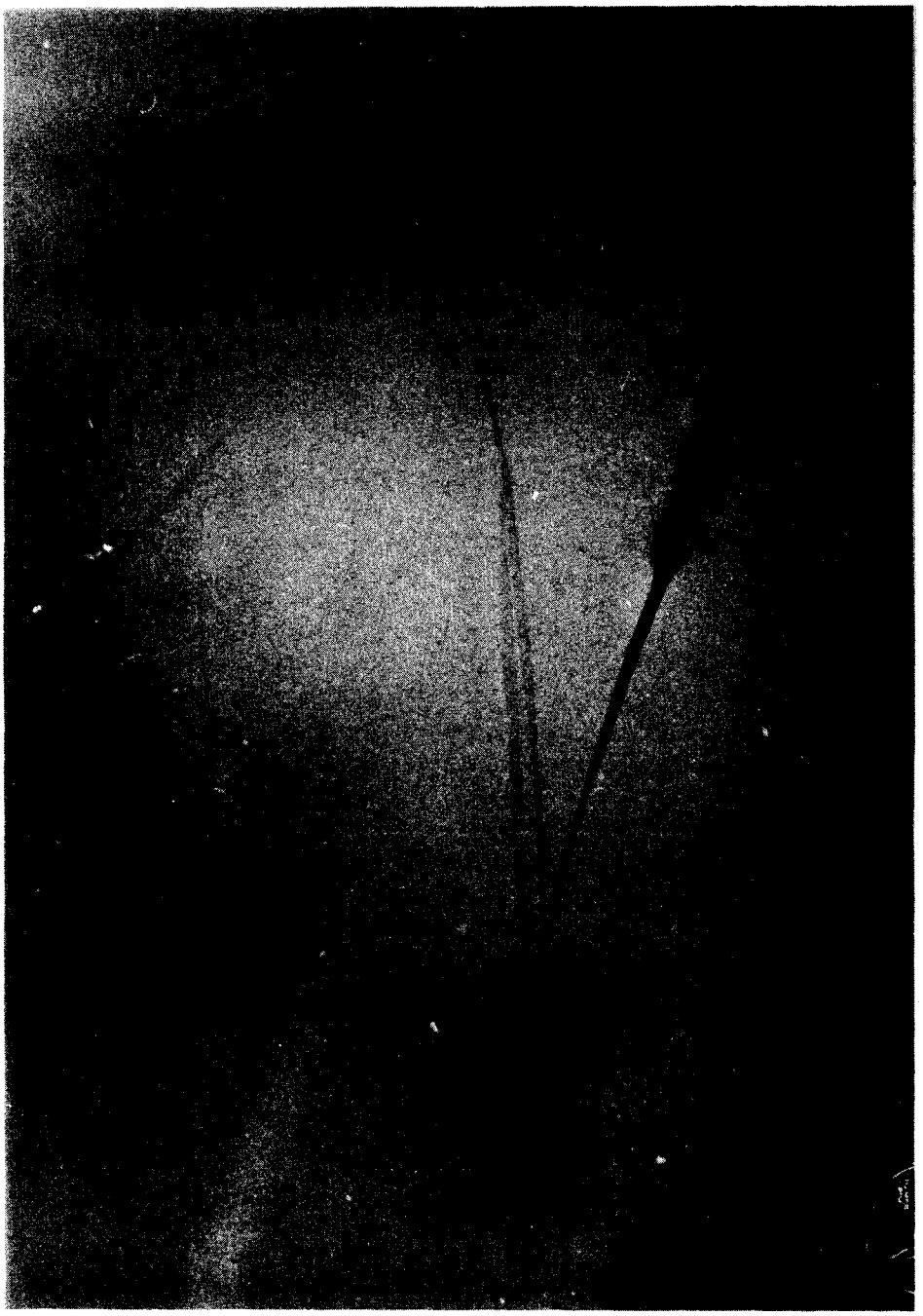


Figure 28. Autoradiograph of Wheat Plant: ^{14}C-Alachlor Applied to the First Leaf

shows a high concentration of what appears to be apoplastic movement of alachlor to the tip of the treated leaf with some downward movement into the roots and up into the younger leaf tissue.

Ten day old soybean plants were treated with ^{14}C-alachlor on one of the primary leaves in 10 one-µl drops. Primary leaf treatment of soybeans with alachlor resulted in almost no movement of the material as shown in Figure 29. Some very slight apoplastic movement within the treated leaf did occur.

Figure 29. Autoradiograph of Soybean Leaf: ^{14}C-Alachlor Applied to a Primary Leaf

Sectioning and Counting of Plant Components

After the autoradiograms were obtained the plants were sectioned into various components, weighed, ground in 95% ethanol and portions of the homogenate were counted. The wheat plants treated through the roots were sectioned in roots, stems, first, second, and third leaf (Figure 30).

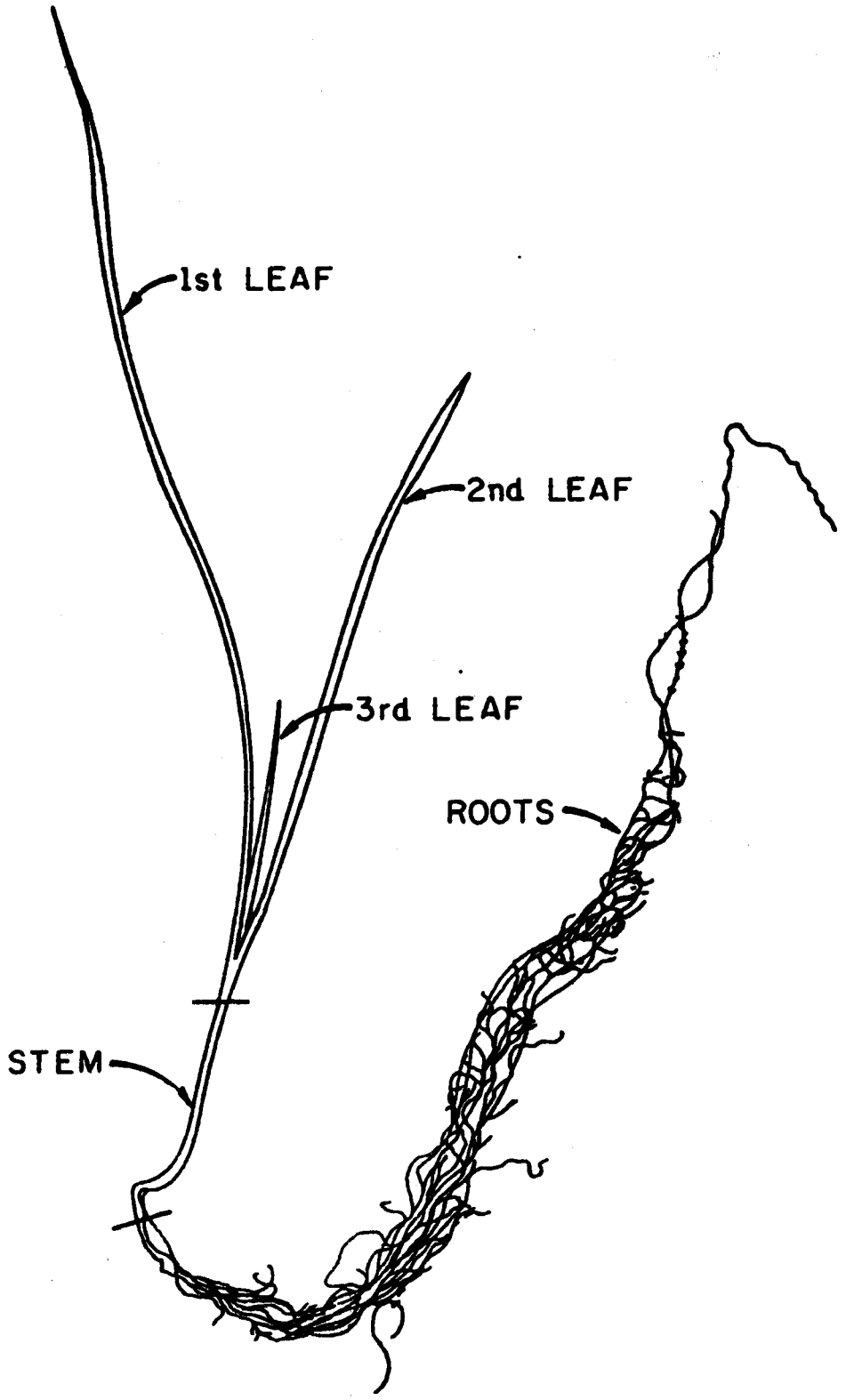


Figure 30. Plant Sectioning of a Wheat Plant: ^{14}C-Alachlor Applied Through the Roots

TABLE VI

DISTRIBUTION OF ROOT APPLIED ^{14}C-ALACHLOR IN WHEAT

Plant Parts	Total DPM	Percentage of Total Applied	DPM X 10^{-3} Per Gram
First Leaf	8,491	0.92	729 b[1]
Second Leaf	6,229	0.68	625 bc
Third Leaf	389	0.04	288 d
Stem	5,594	0.61	502 c
Roots	89,448	9.71	5,462 a
Nutrient Solution	734,504	79.70	---

[1]Numbers followed by the same letter are not significantly different at the 0.01 level.

The small letters in Table VI indicate differences as determined by Duncan's multiple range test at the 1% level. The greatest accumulation of alachlor in the wheat plants occurred in the roots with the first or oldest leaf containing more alachlor than the younger leaf tissue. There was 10 percent of the ^{14}C-alachlor that could not be accounted for, 10 percent in the plant with the bulk of the material in the roots and approximately 80 percent of the material applied remained in the nutrient solution. Part of this loss could have occurred when the roots were washed for one minute with water after being removed from the treatment solution. Additional loss may have occurred during the 48 hour treatment period when air was bubbled through the treatment solution for aeration of the wheat roots.

Soybean plants treated through the roots were sectioned into roots, stem, cotyledons, primary leaves, trifoliate leaf and growing point (Figure 31).

TABLE VII

DISTRIBUTION OF ROOT APPLIED ^{14}C-ALACHLOR IN SOYBEANS

Plant Parts	Total DPM	Percentage of Total Applied	DPM X 10^{-3} Per Gram
Growing Point	1,214	0.13	38 e[1]
Trifoliate Leaves	15,385	1.71	112 c
Primary Leaves	21,008	2.33	168 b
Cotyledons	1,962	0.22	46 e
Stem	5,488	0.61	65 d
Roots	486,535	54.01	4,886 a
Nutrient Solution	841,035	34.35	---

[1]Numbers followed by the same letter are not significantly different at the 0.01 level.

In soybeans the largest concentration of alachlor was found in the roots with the primary leaves having the second largest amount (Table VII). The primary leaves are older tissue than either the trifoliate leaf or the growing point. The stem contained more alachlor than the cotyledons or the growing point with the growing point containing the least amount of alachlor. Approximately 35 percent of the

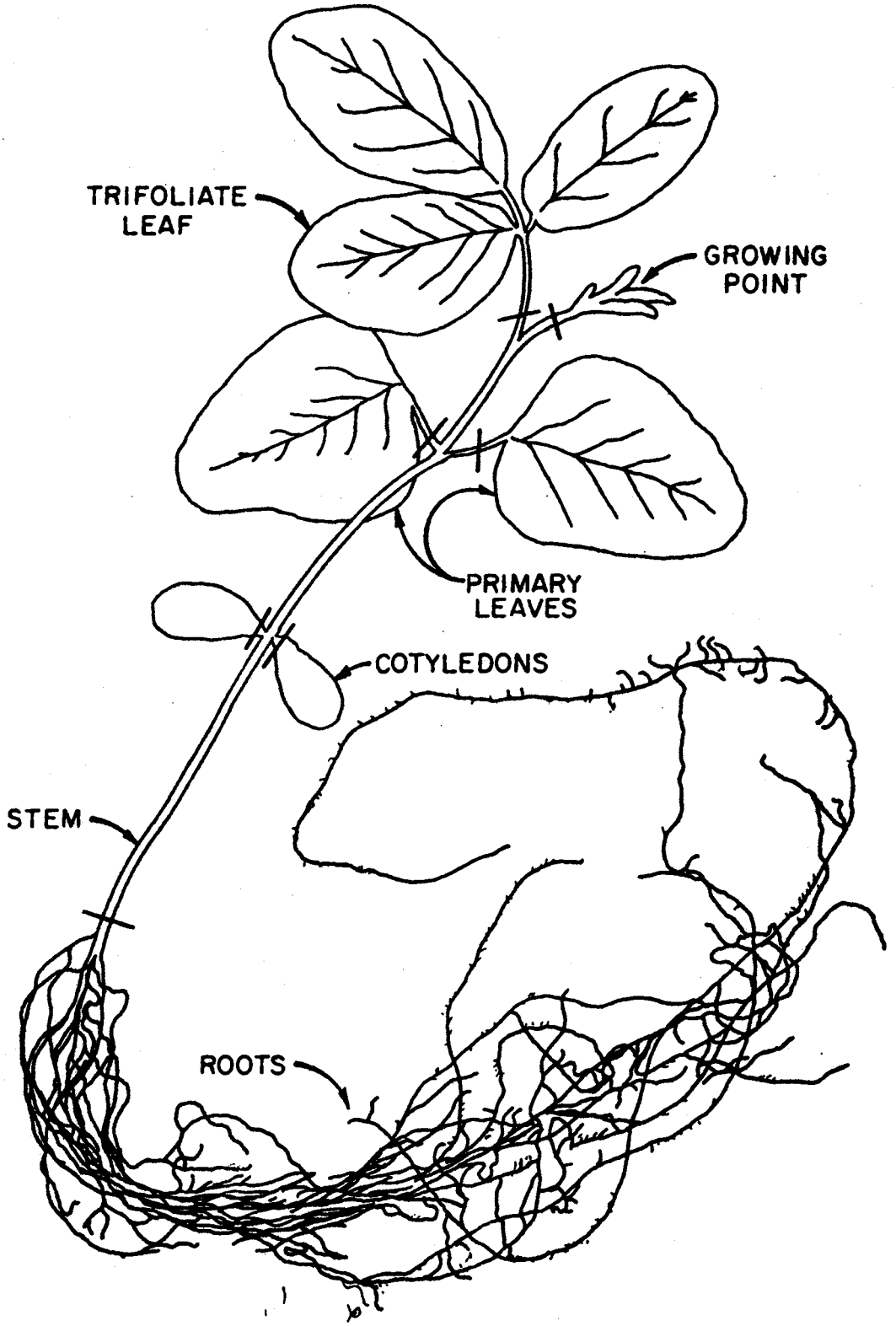


Figure 31. Plant Sectioning of a Soybean Plant: ^{14}C-Alachlor Applied Through the Roots

material applied remained in the nutrient solution while 60 percent was in the plant and a loss of 5 percent occurred. The loss probably occurred in a similar manner to that in wheat.

The same concentration of alachlor was added to both wheat and soybean roots, but due to the smaller size of the wheat roots there was less total uptake of alachlor for the wheat. Samples removed from the nutrient solution at 4, 16, 28, 40 and 48 hours show a depletion of ^{14}C-alachlor with an increase in time. When the data was expressed on a per gram fresh weight basis the wheat took up more alachlor than the soybeans as denoted by the greater slope of the curve of data for wheat over the data for soybeans (Figure 32).

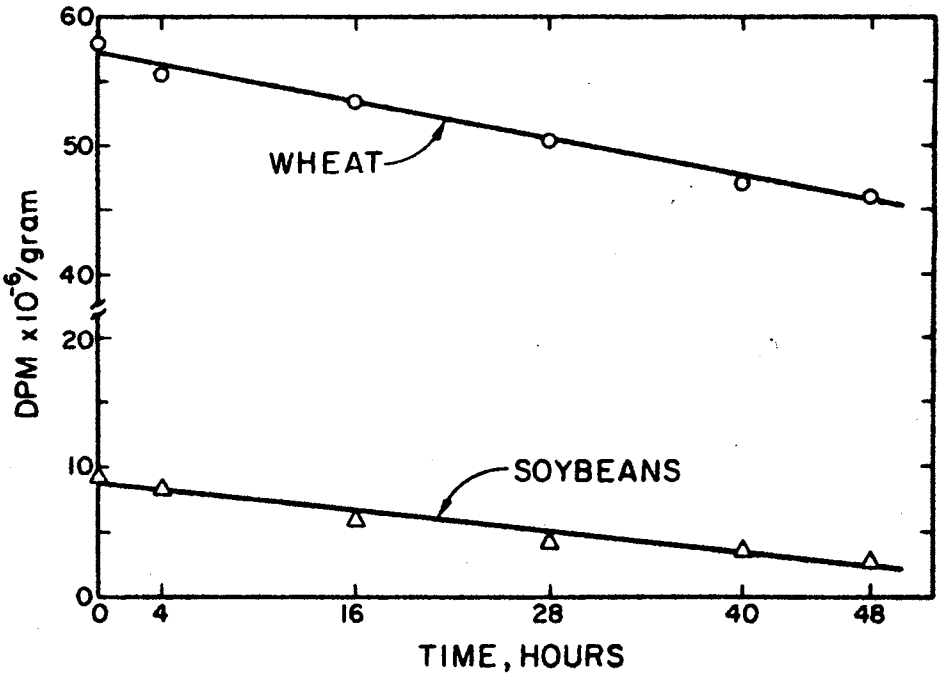


Figure 32. Depletion of ^{14}C-Alachlor from the Nutrient Solution of Wheat and Soybeans

TABLE VIII

DISTRIBUTION OF LEAF APPLIED ^{14}C-ALACHLOR IN WHEAT

Plant Parts	Total DPM	Percentage of Total Applied	DPM X 10^{-3} Per Gram
First Leaf			
Leaf Tip	61,098	11.10	20,081 b[1]
Treated Tip	268,399	48.76	49,991 a
Leaf Base	1,043	0.18	138 c
Second Leaf	240	0.04	20 ef
Third Leaf	448	0.08	35 d
Stem	305	0.06	21 e
Roots	429	0.08	15 f
Nutrient Solution	3,568	0.65	---

[1]Numbers followed by the same letter are not significantly different at the 0.01 level.

Wheat plants treated on primary leaf tissue were sectioned in roots, stem, third leaf, second leaf and the first leaf was divided into leaf base, treated area and leaf tip (Figure 33). Grinding and then liquid scintillation counting confirms the autoradiographs in that there is a very high accumulation of alachlor remaining in the treated area with movement into the leaf tip of the treated leaf (Table VIII). There was some movement downward in the treated leaf into the stem, roots and the younger leaf tissue. In tissue other than the treated leaf there occurred a significant accumulation in the third or youngest leaf tissue. Approximately 50 percent of the material applied remained on the

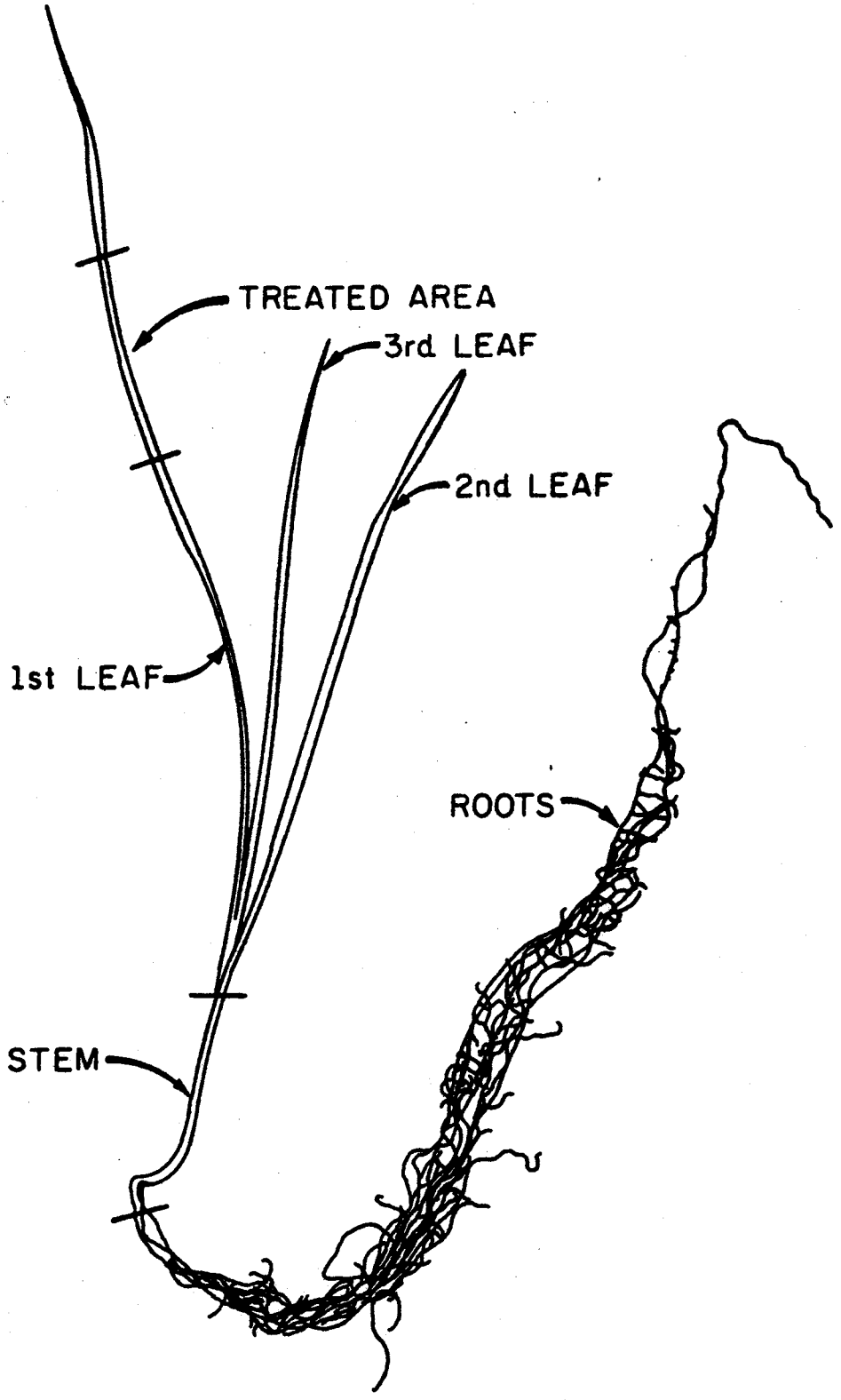


Figure 33. Plant Sectioning of a Wheat Plant: ^{14}C-Alachlor Applied to the First Leaf

treated leaf for which only 61 percent of the total was accounted. The loss was thought to occur from the treated tissue during the freeze drying process and possibly during the 48 hour treatment period from the leaf surface.

Soybean plants treated on primary leaf tissue were sectioned into roots, stem, cotyledons, treated primary leaf, non-treated primary leaf, trifoliate leaf and growing point (Figure 34). The distribution of ^{14}C-alachlor in soybeans entering through a primary leaf was very limited with slight accumulations occurring only in the cotyledons and the growing point (Table IX).

TABLE IX

DISTRIBUTION OF LEAF APPLIED ^{14}C-ALACHLOR IN SOYBEANS

Plant Parts	Total DPM	Percentage of Total Applied	DPM X 10^{-3} Per Gram
Growing Point	240	0.04	27 b[1]
Trifoliate Leaves	1,991	0.36	8 c
Untreated Primary Leaf	734	0.13	7 c
Treated Primary Leaf	377,403	68.56	5,307 a
Cotyledons	754	0.14	16 b
Stem	640	0.12	6 c
Roots	960	0.17	7 c
Nutrient Solution	3,052	0.55	---

[1]Numbers followed by the same letter are not significantly different at the 0.05 level.

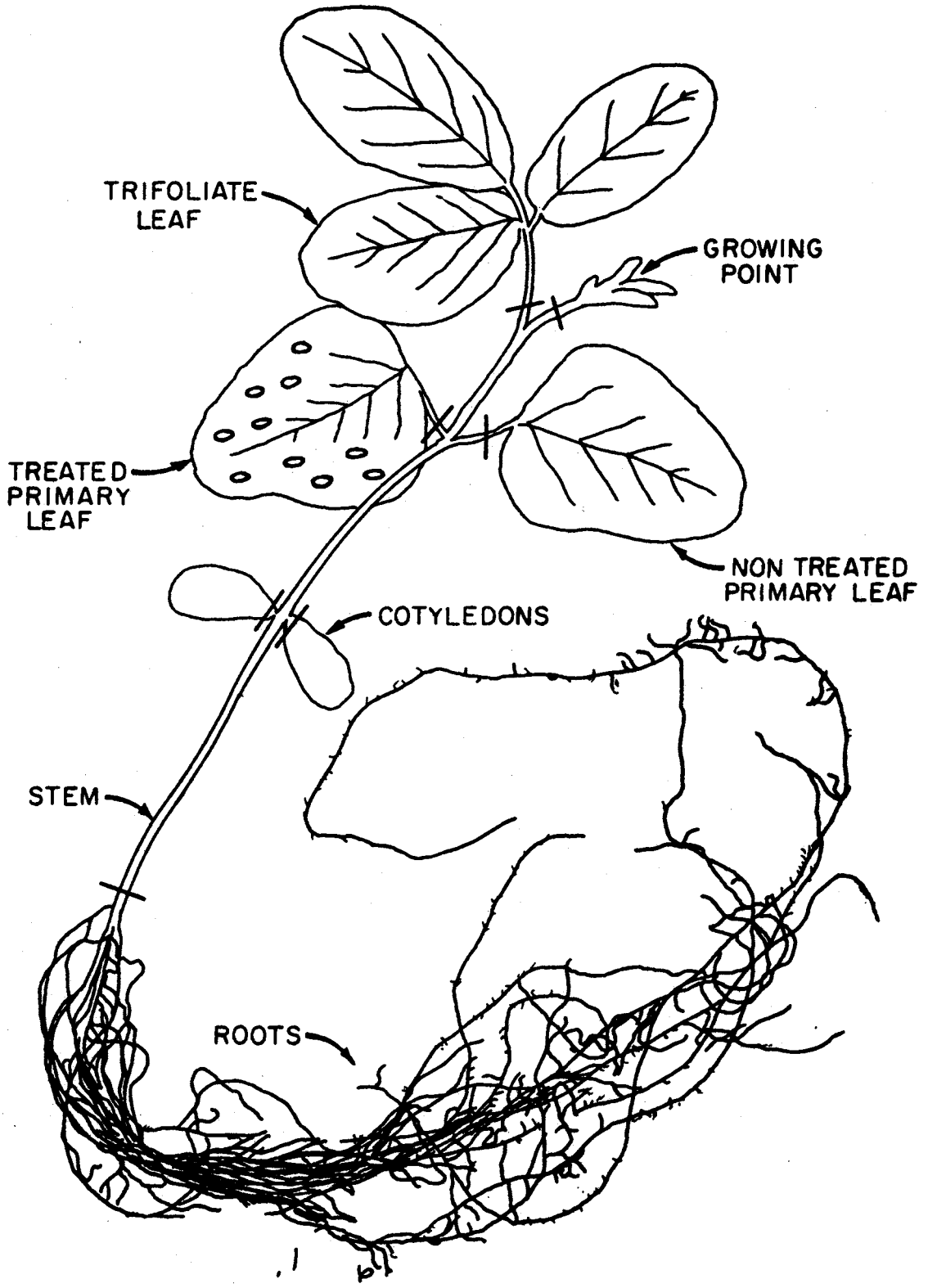


Figure 34. Plant Sectioning of a Soybean Plant: ^{14}C-Alachlor Applied to a Primary Leaf

Approximately 69 percent of the total alachlor applied remained on or in the treated leaf with only one percent accounted for in the rest of the plant and nutrient solution. Losses encountered probably occurred in a manner similar to those in wheat.

The exudation of ^{14}C-alachlor into the nutrient solution on a per gram dry weight basis for both wheat and soybeans treated through the leaf showed a rhythmic fluctuation over a 48 hour period (Figure 35). Greater fluctuation or movement of alachlor was observed in the wheat species.

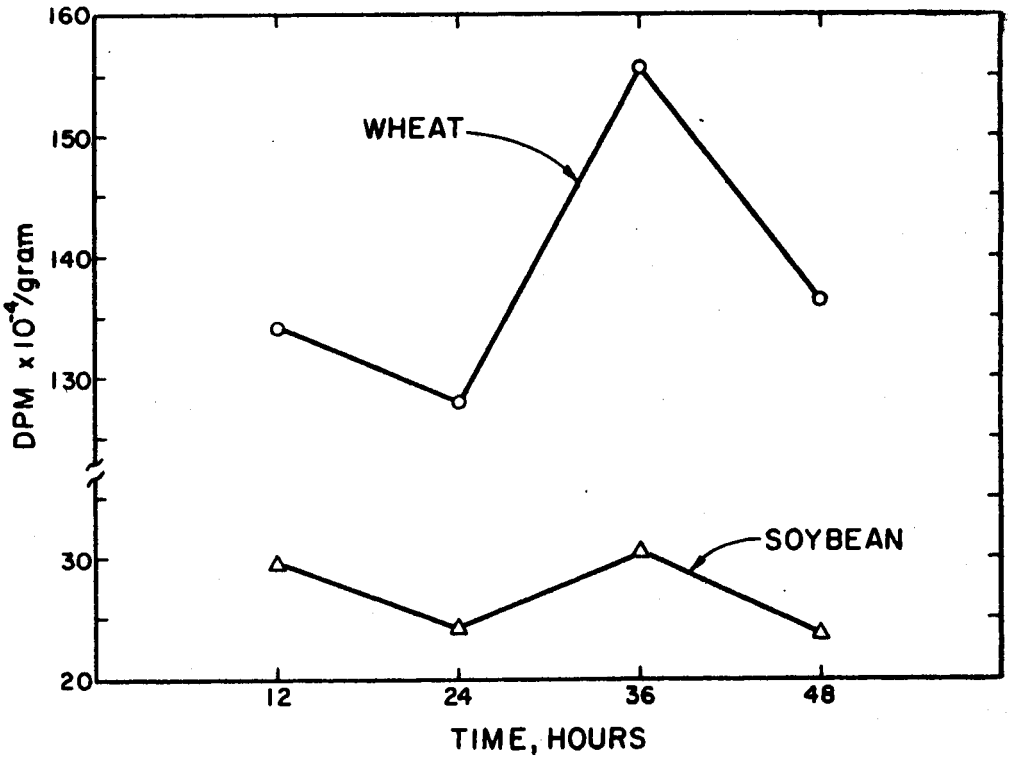


Figure 35. Appearance of ^{14}C-Alachlor in the Nutrient Solution of Wheat and Soybean Plants Treated on a Primary Leaf

Plant Tissue Uptake

Weed seedlings germinating and emerging in a silica sand were treated preemergence with alachlor. Alachlor was quite active in the coleoptile region of moncots such as wheat. The coleoptile became brittle and quite rigid and did not allow the shoot to extend through it. Frequently the shoot splits the coleoptile at the base and emerges. This indicated that the coleoptile was either sensitive to alachlor or tended to accumulate alachlor more than other tissue.

With the above in mind, a study was set up to look at alachlor uptake by vairous types of excised wheat tissue. The root tissue reached a maximum concentration of alachlor at 4 hours and leveled off while both the coleoptile and leaf tissue continued to take up alachlor after 32 hours under both light and dark conditions (Figure 36). Uptake of alachlor by both leaf and coleoptile tissue in the light was significantly greater than that in the dark, but there were no differences in total accumulation between leaves and coleoptiles. Leaves and coleoptiles accumulated much higher concentrations of alachlor than roots.

From the above studies it is quite apparent that alachlor uptake and translocation varies with species, plus location of application.

Alachlor application to the roots of soybean and wheat resulted in complete movement throughout both species. The roots of both species accumulated the greatest amount of alachlor while the youngest or most actively growing foliar tissue contained the least amount of alachlor. In the above ground tissue there was greater movement and accumulation in the older foliar tissue. On a per gram basis the uptake of alachlor was greater in wheat than in the soybeans. The depletion of alachlor from the nutrient solution on a per gram basis also showed a greater

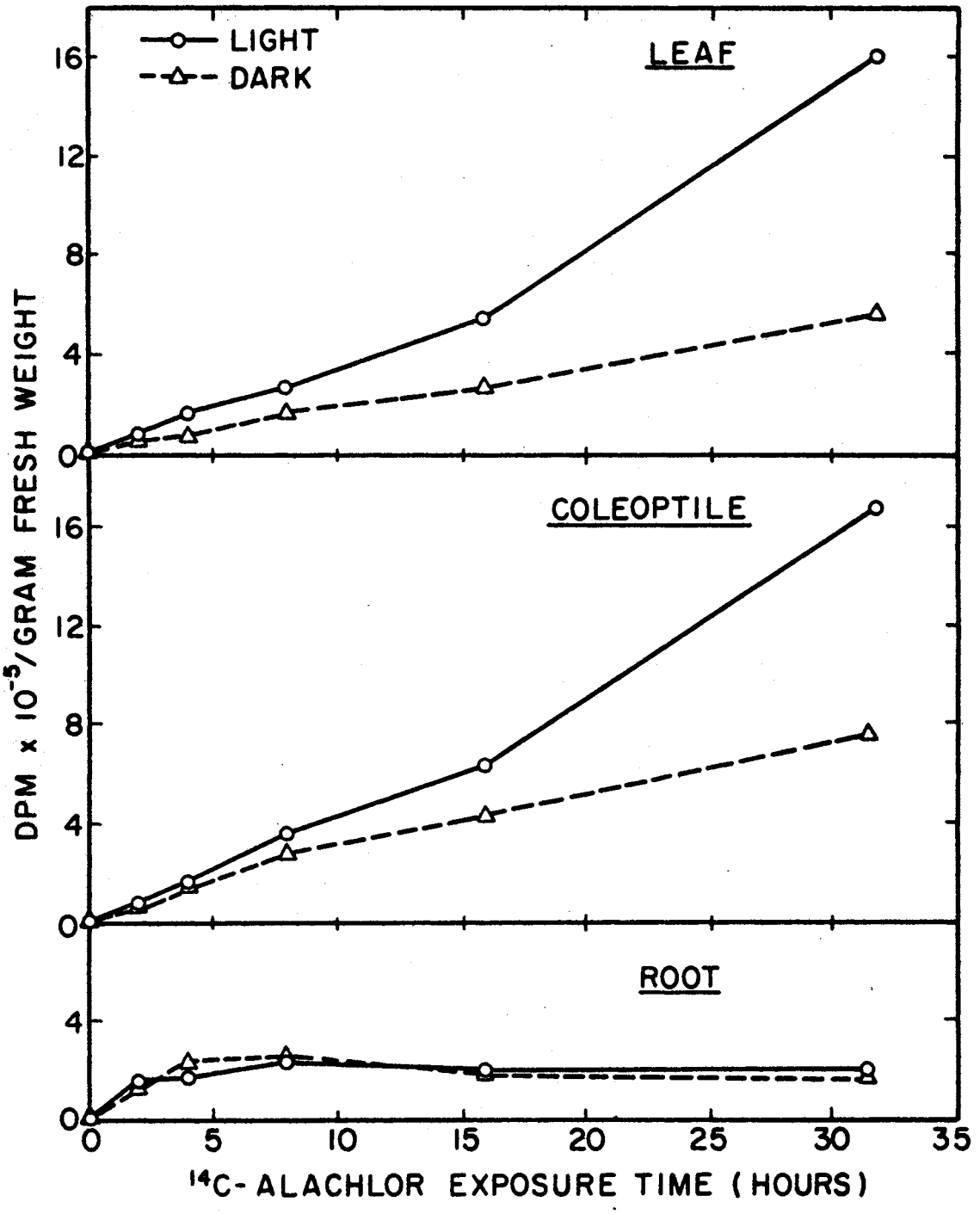


Figure 36. Total Uptake of ^{14}C-Alachlor by Wheat Tissue

uptake of alachlor by wheat than soybeans.

The greater uptake of alachlor by wheat helps explain the greater susceptibility of wheat found in the field and biossay studies as compared to the more resistant species, soybeans, with less uptake of alachlor.

The uptake and movement of alachlor from treated primary leaf tissue of wheat and soybeans was very limited. The majority of the alachlor applied to the primary leaf tissue of both wheat and soybeans remained in the treated leaf with significant apoplastic movement into the tip of the treated wheat leaf occurring.

In wheat there was some very slight movement into the non-treated foliar tissue and roots while almost no movement from the treated tissue occurred in soybeans.

On a per gram basis there was more exudation of alachlor into the nutrient solution by wheat than soybeans. The rhythmic fluctuation of alachlor in the nutrient solution occurred with both species, but the movement in wheat was much greater than in soybeans.

As with the root treatment there was more movement in wheat than in soybeans where almost no movement occurred. The foliar treatment data shows the importance not only of alachlor uptake but also movement within the plants as related to the degree of resistance and susceptibility.

In the wheat tissue uptake study the leaf and coleoptile tissue accumulated more alachlor than the root tissue. This helps explain why alachlor, in Knake and Wax's (10) study, was more effective in the shoot zone than in the seed zone.

CHAPTER V

SUMMARY

Field, greenhouse, growth chamber and laboratory studies were conducted to gain a better understanding of the herbicidal activity and selectivity of alachlor.

In the field it was found that soybeans were highly resistant to alachlor while cotton and morningglory were moderately resistant. Brachiaria, Texas panicum and prickly sida were slightly resistant while wheat, oats and foxtail millet were moderately susceptible. Crabgrass and spiny pigweed were both highly susceptible to alachlor.

Bioassay studies showed wheat to be the best indicator species to a wide range of alachlor rates. Crabgrass was found to be an excellent indicator of alachlor at concentrations less than one ppm. The postemergence activity of alachlor on soybeans and sorghum was almost nonexistent.

Inhibition of the Hill reaction in isolated wheat chloroplasts from 14 or 21 day old Kaw wheat plants did not occur with the addition of alachlor at 5×10^{-4}, 5×10^{-5} or 5×10^{-6} M concentrations.

In general, plants treated with alachlor and receiving a nitrogen or no nitrogen pretreatment were not significantly different in nitrate content or nitrate reductase activity.

It was found that alachlor may either stimulate or inhibit both nitrate uptake and nitrate reductase activity, depending on the

concentration used, order of exposure, or the total time of exposure. The nitrate content was significantly reduced by both concentrations of alachlor or by an increase in total exposure time to alachlor. Alachlor was less inhibitory if added during the first half of a 15 hour induction period than in the second half. Nitrate reductase activity was inversely proportional to alachlor exposure time.

The high concentration of alachlor caused a significant reduction in amino acid content while the lower concentration did not. The addition of alachlor for at least 8 hours at the beginning of induction caused a significant reduction in protein content.

Exposure of plants to alachlor concentrations of 5×10^{-5} or 5×10^{-6} M for 4 hours before replacement with nitrate caused a significant stimulation of nitrate uptake, while addition for longer than 8 hours reduced uptake. Additions of alachlor following nitrate caused a signigicantly greater reduction in nitrate uptake than the addition of alachlor before nitrate.

A stimulation of nitrate reductase activity occurred with 4 hours of alachlor following 12 hours of nitrate. An increased time exposure of alachlor after nitrate caused a significant reduction in nitrate reductase activity. The nitrate ion concentration at 8 hours was high enough to support nitrate reductase activity much higher than was observed. This indicates the inhibition of nitrate reductase activity by alachlor. Generally, as the total time exposure of alachlor was increased, the amino acid content decreased. The high concentration of alachlor caused a significant reduction in protein content while the low concentration did not.

In soybeans and wheat, the application of ^{14}C-alachlor for 48 hours

to the roots resulted in greater accumulation in the roots while the youngest or most actively growing foliar tissue contained the least amount of alachlor. On a per gram basis, the uptake of alachlor was greater in wheat than in soybeans.

The uptake and movement of alachlor from treated primary leaf tissue of wheat and soybeans was very limited. The majority of the alachlor applied to the primary leaf tissue of both wheat and soybeans remained in the treated leaf with highly significant apoplastic movement into the tip of the treated wheat leaf. In both species there was some very slight movement into the non-treated foliar tissue and roots.

The exudation of alachlor into the nutrient solution of both wheat and soybeans treated through a primary leaf shows a rhythmic fluctuation over the 48 hour treatment period with the greatest movement occurring in the wheat. On a per gram basis there was more exudation of alachlor into the nutrient solution by wheat than soybeans.

The uptake of alachlor by wheat tissue shows that root tissue reaches a maximum concentration of alachlor at 4 hours and levels off while both the coleoptile and leaf tissue continued to increase in uptake after 32 hours. Uptake of alachlor by both leaf and coleoptile tissue in the light was significantly greater than that in the dark.

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APPENDIX

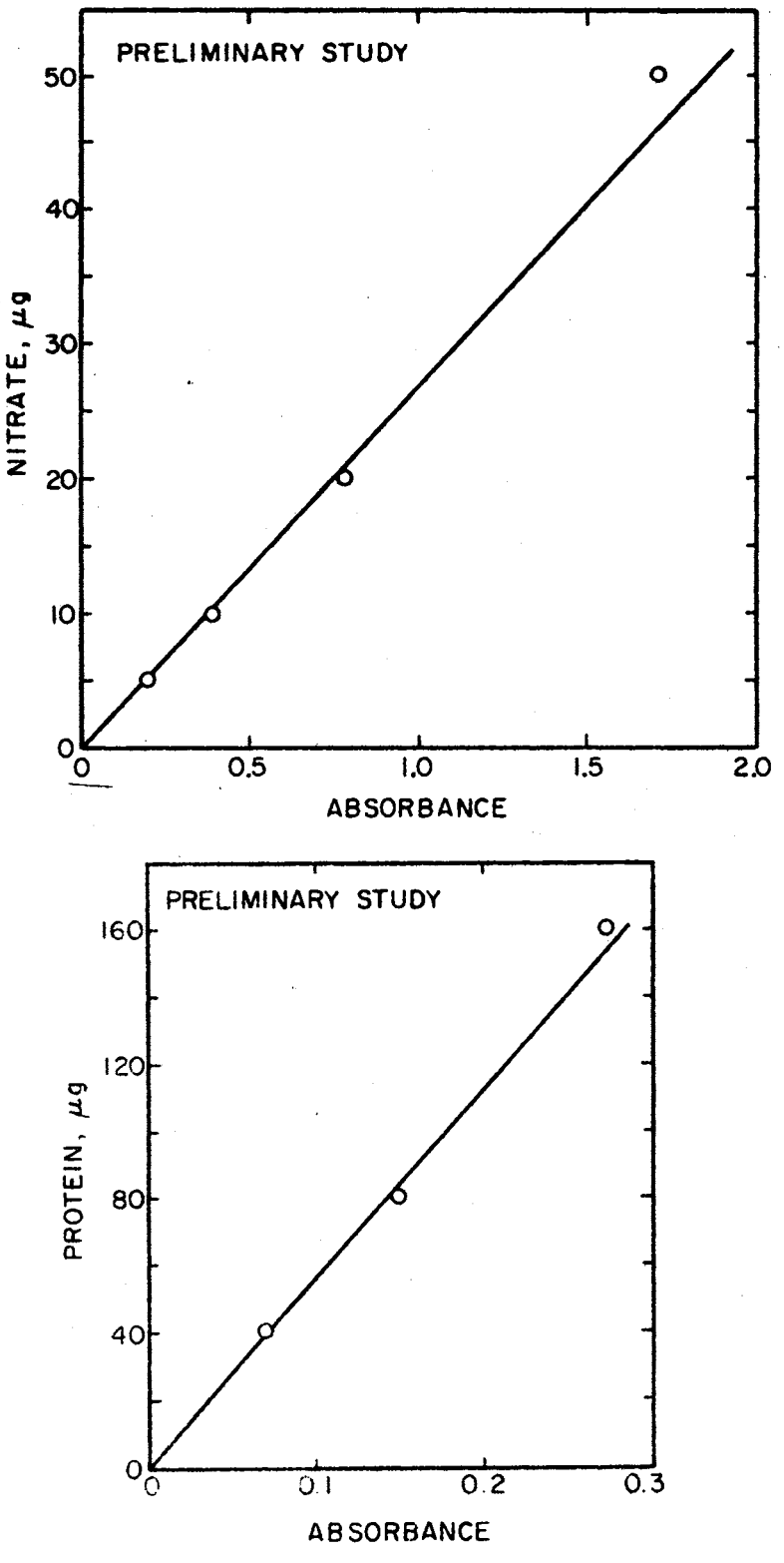

Figure 37. Nitrate and Protein Standard Curves (Preliminary Study)

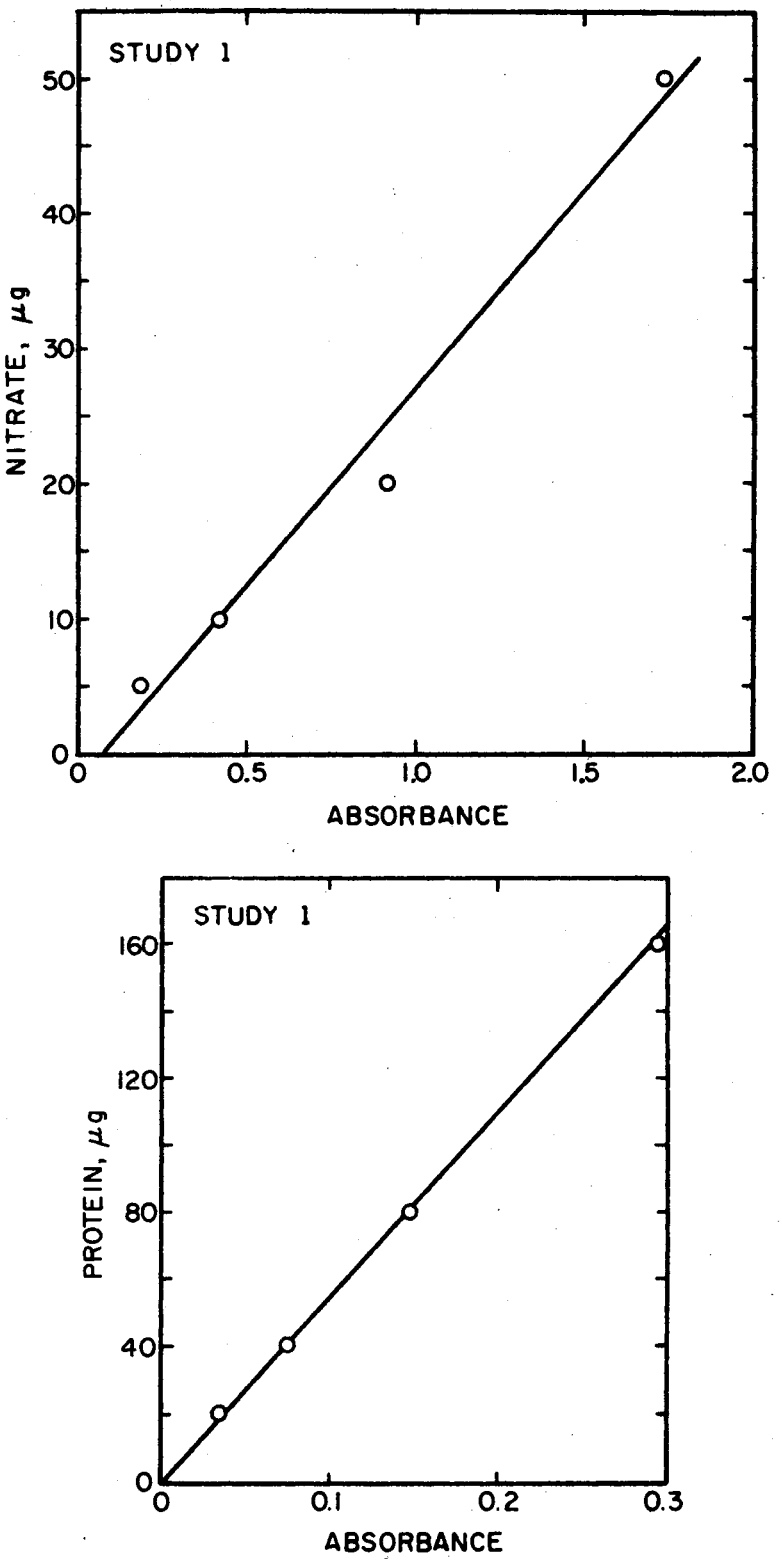

Figure 38. Nitrate and Protein Standard Curves (Study 1)

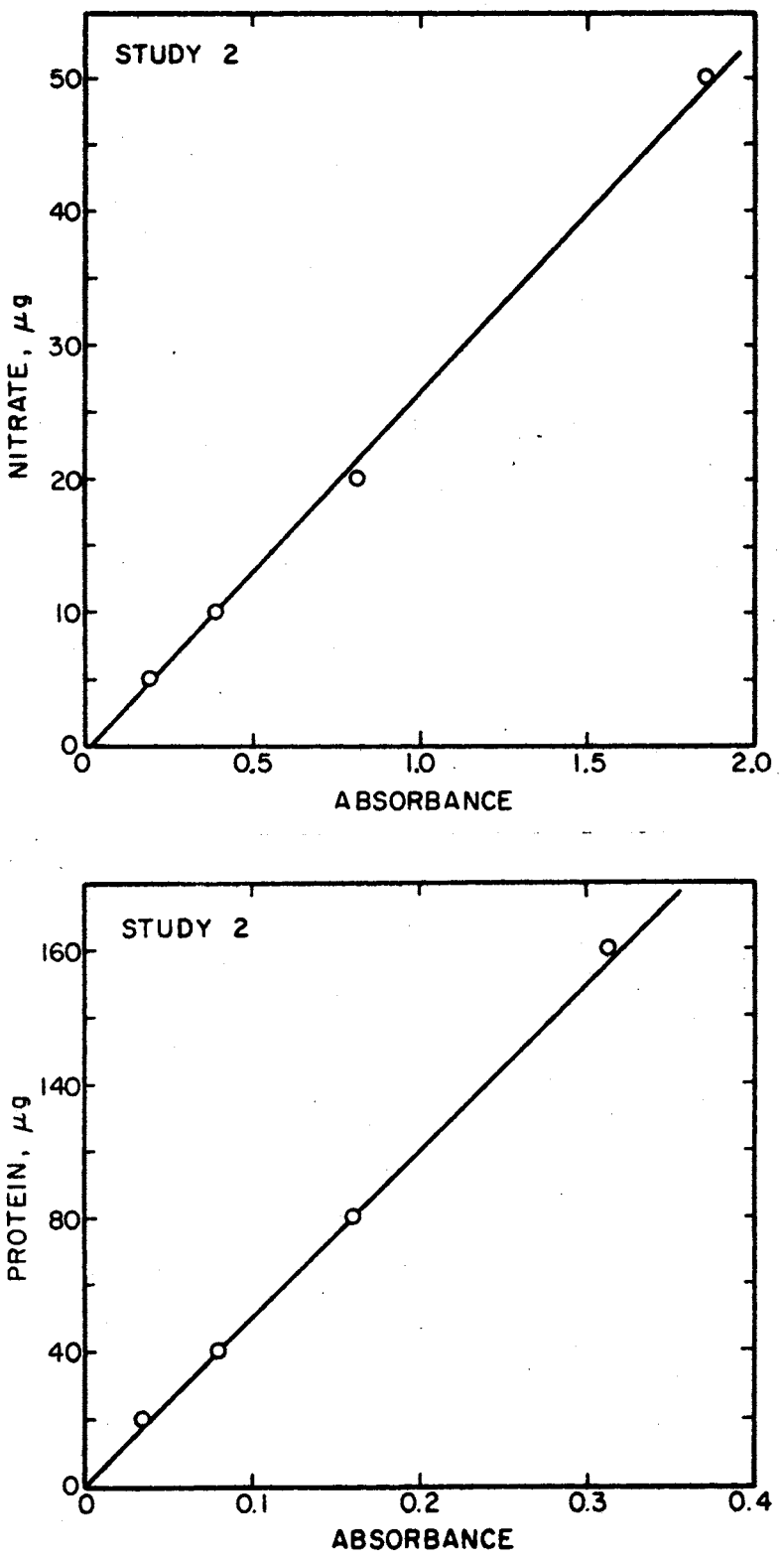

Figure 39. Nitrate and Protein Standard Curves (Study 2)

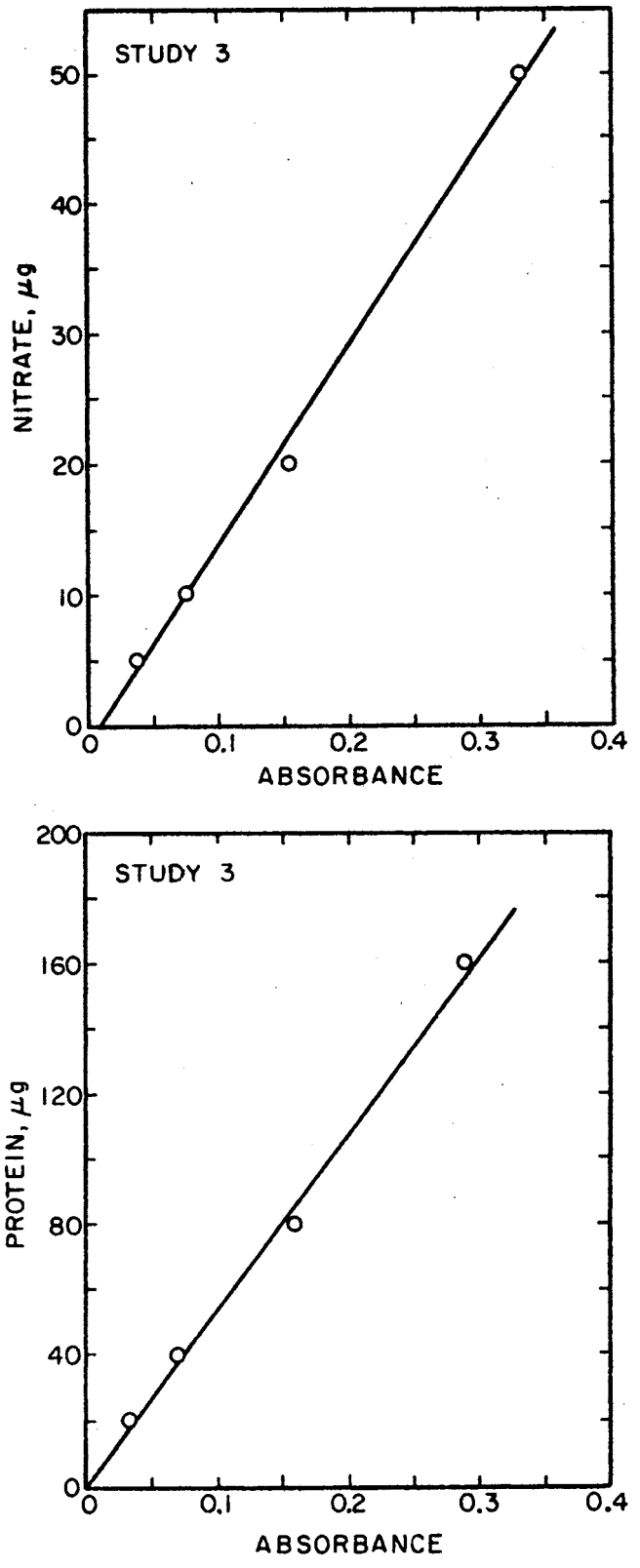

Figure 40. Nitrate and Protein Standard Curves (Study 3)

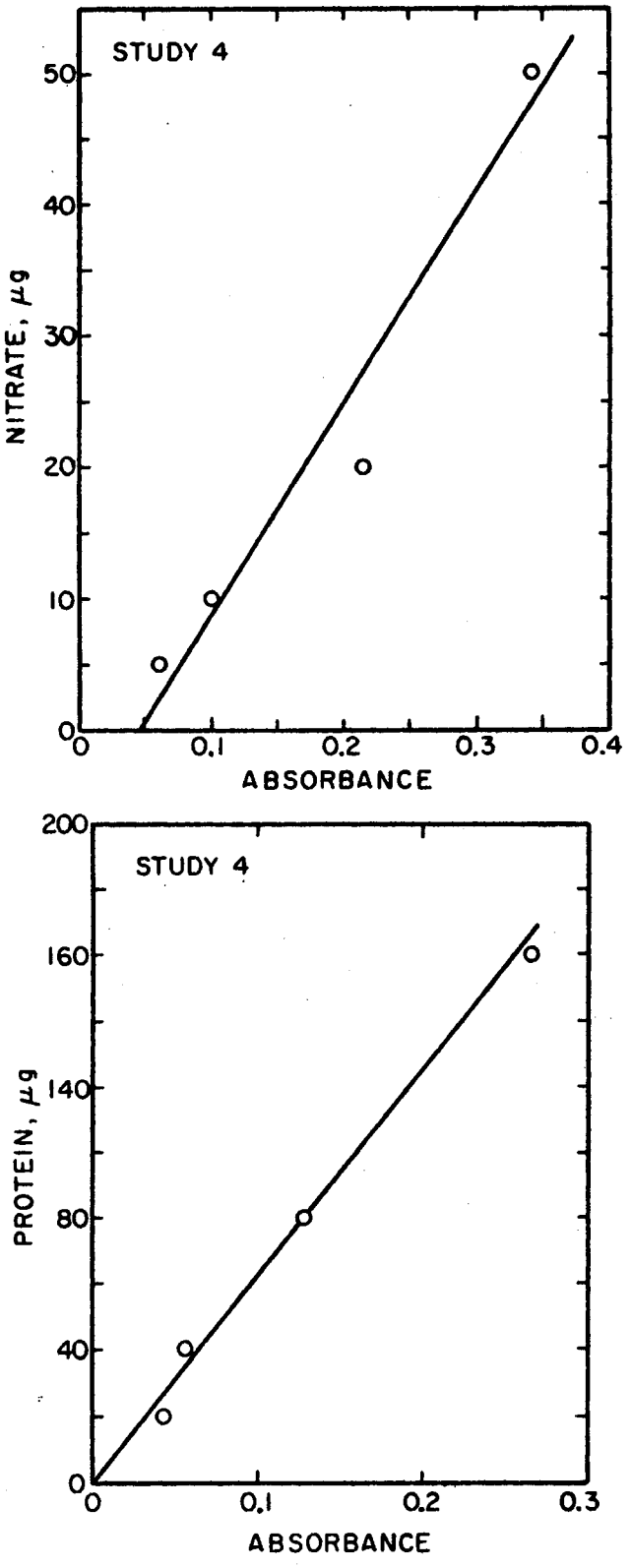

Figure 41. Nitrate and Protein Standard Curves (Study 4)

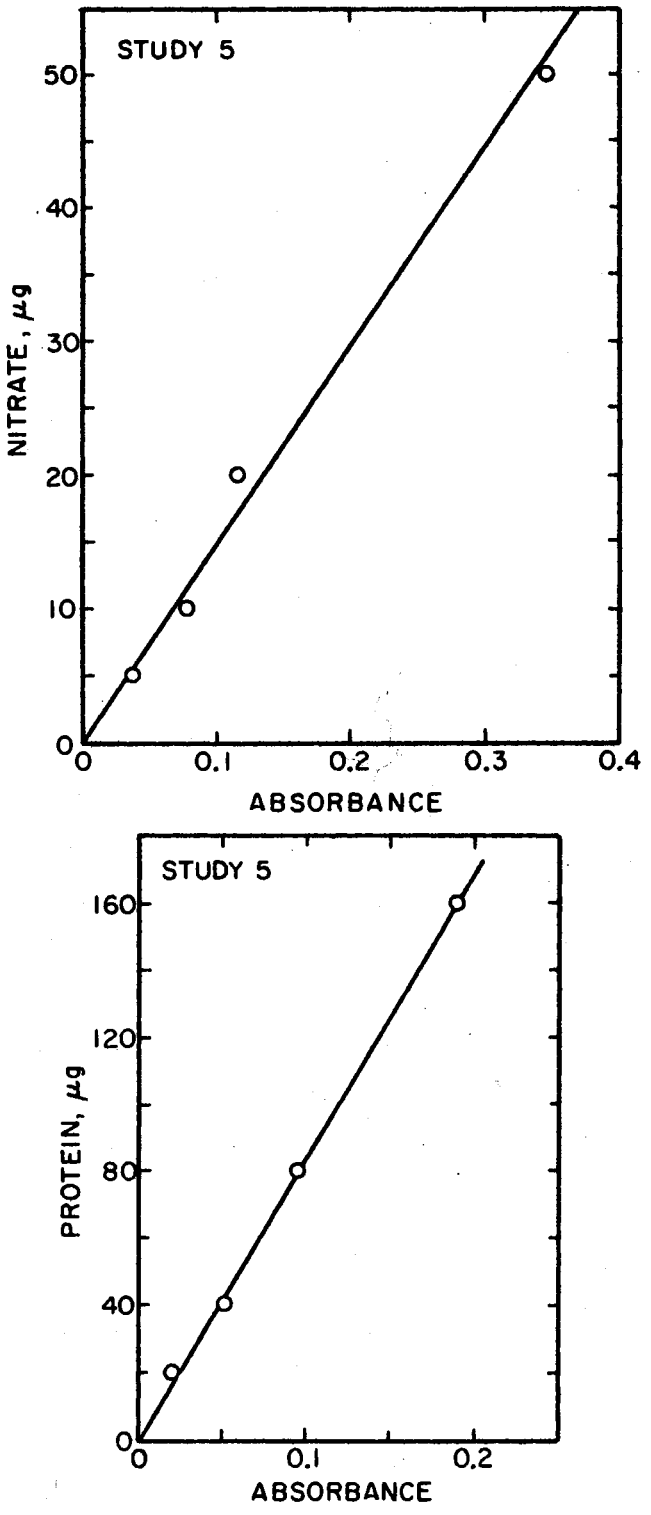

Figure 42. Nitrate and Protein Standard Curves (Study 5)

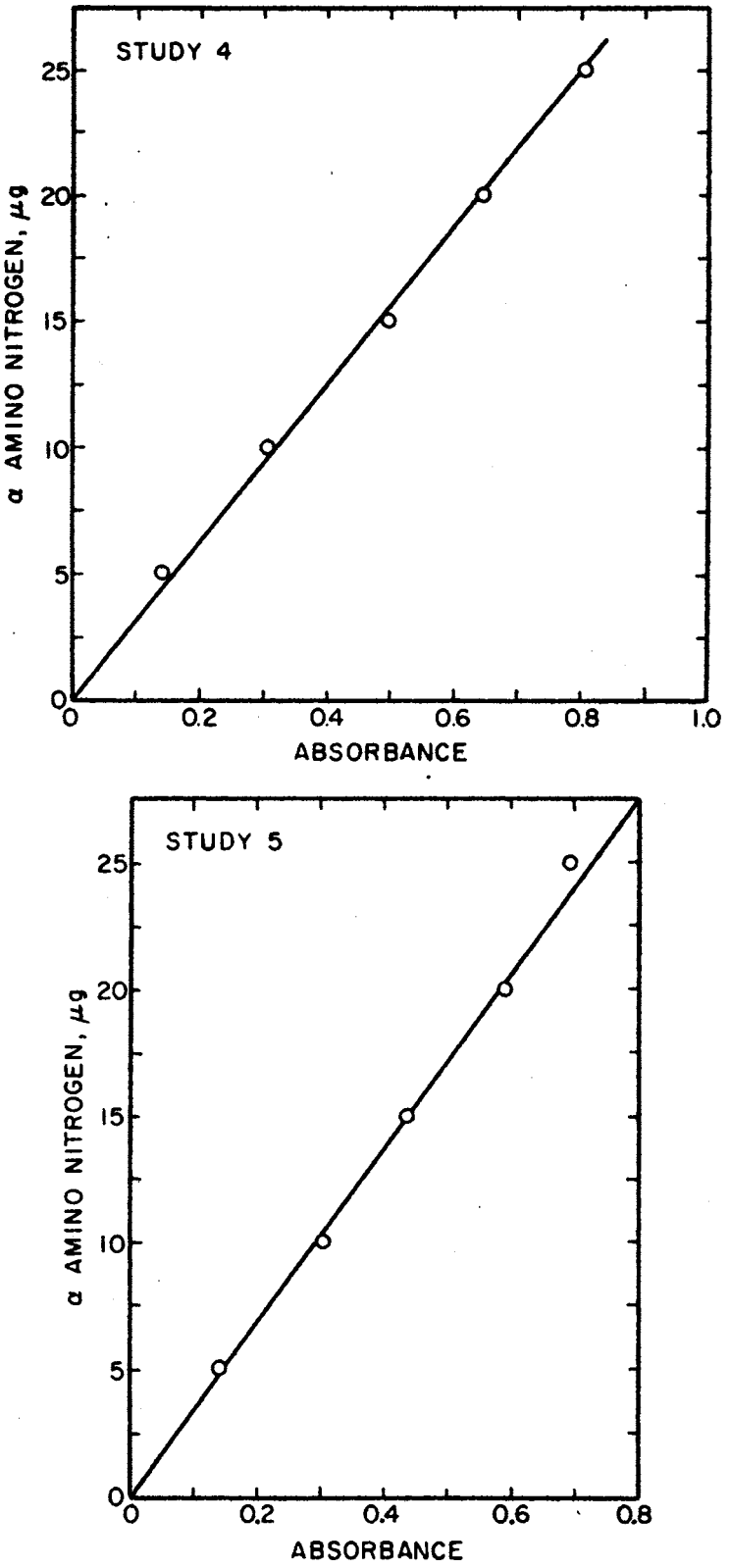

Figure 43. Amino Acid Standard Curves (Studies 4 and 5)

TABLE X

EFFECTS OF ALACHLOR ON THE HILL REACTION OF ISOLATED WHEAT CHLOROPLAST (STUDY 1)

Klett Units	Alachlor Concentrations (molar)			
Time (sec).	0	5×10^{-6}	5×10^{-5}	5×10^{-4}
0	0.400	0.419	0.415	0.437
30	0.323	0.338	0.339	0.359
60	0.248	0.266	0.260	0.289
90	0.192	0.205	0.198	0.218
120	0.155	0.143	0.149	0.157
150	0.137	0.141	0.142	0.141
180	0.137	0.141	0.142	0.141

TABLE XI

INHIBITION OF THE HILL REACTION OF ISOLATED WHEAT CHLOROPLAST BY ALACHLOR AND DIURON (STUDY 2)

Klett units	Alachlor conc. (molar)		Diuron conc. (molar)
Time (sec.)	0	5×10^{-4}	5×10^{-4}
0	0.335	0.345	0.360
30	0.292	0.321	0.357
60	0.262	0.267	0.357
90	0.227	0.228	0.357
120	0.191	0.197	0.357
150	0.161	0.158	0.357
180	0.132	0.129	0.357
210	0.104	0.103	0.357

TABLE XII

EFFECTS OF ALACHLOR ON THE HILL REACTION OF ISOLATED WHEAT CHLOROPLAST (STUDY 3)

Klett units	Alachlor concentration			
Time (sec.)	0	5×10^{-6}	5×10^{-5}	5×10^{-4}
0	0.406	0.400	0.398	0.397
30	0.303	0.307	0.306	0.300
60	0.233	0.238	0.236	0.216
90	0.180	0.179	0.180	0.155
120	0.136	0.136	0.136	0.131
150	0.313	0.133	0.133	0.131

VITA

James Michael Chandler

Candidate for the Degree of

Doctor of Philosophy

Thesis: PHYSIOLOGICAL ASPECTS OF THE HERBICIDAL ACTIVITY OF ALACHLOR

Major Field: Crop Science

Biographical:

Personal Data: Born September 30, 1943, at Wichita, Kansas, the son of James O. and Louise O. Chandler.

Education: Attended grade school in Seminole, Oklahoma; graduated from Palo Duro High School, Amarillo, Texas in 1961; received the Bachelor of Science degree from West Texas State University with a major in Agronomy in May, 1965; received the Master of Science degree from Oklahoma State University, with a major in Agronomy, in May, 1968; completed requirements for the Doctor of Philosophy degree in May, 1971, majoring in Crop Science.

Professional Experience: Worked for Dr. Kenneth Porter, wheat breeder for Texas Agriculture Experiment Station in Bushland, Texas in 1961; worked for Dr. Allen F. Wiese, Weed Research Specialist for Texas Agriculture Experiment Station in Bushland, Texas, full time the summers of 1962-1964 and half time the winters of 1961-1964; taught beginning Crops Lab in 1962 and 1963 for Agronomy Department at West Texas State University, Canyon, Texas; served as graduate research assistant while working on the Master of Science degree from Oklahoma State University; recipient of an NDEA Fellowship for 1968-1970 and taught the Weed Science Lab 1969-1970 at Oklahoma State University while completing requirements for the Doctor of Philosophy degree in Crop Science.

Member of: Who's Who in American Colleges and Universities, Alpha Chi National Honorary Society, Beta Beta Beta National Biological Fraternity, American Society of Agronomy, Sigma Xi, and Weed Science Society of America.