THE INFLUENCE OF NUTRIENT LEVEL AND COMBINATION ON HERBICIDE UPTAKE AND PHYTOTOXICITY

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ABSTRACT

THE INFLUENCE OF NUTRIENT LEVEL AND COMBINATION ON HERBICIDE UPTAKE AND PHYTOTOXICITY

By

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The influence of nutrients and solution pH on herbicide absorption and phytotoxicity was studied. The phytotoxicity of 3-amino-2,5 dichlorobenzoic acid (amiben), 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine), and 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) in various nutrient combinations and levels was determined in bioassay experiments. Oats (Avena sativa L.) were grown 3 weeks in sand culture with three levels of N, P and K. Injury ratings and shoot dry weights showed that only N interacted with the herbicides. Nitrogen, supplied as nitrate, increased the phytotoxicity of each herbicide. No interactions with P and K were observed.

Herbicide absorption was measured in nutrient solutions containing different N, P and K concentrations and ^{14}C-labeled amiben, linuron and atrazine. Intact corn, (Zea mays L.), soybean (Glycine max Merr.), and pigweed

(Amaranthus retroflexus L.) roots were immersed in treatment solutions 4 hr. The effect of N sources and levels and solution pH on amiben absorption by pigweed and corn roots was determined. Intact corn coleoptiles were similarly treated to compare uptake in roots with that of shoots.

The only consistent nutrient effect on herbicide absorption was a decrease in amiben uptake caused by 10 and 50 mM NO_3^-. This means the increased phytotoxicity of amiben, atrazine, and linuron with increasing nitrate levels observed in the bioassay experiments was not a result of greater herbicide absorption.

Only corn root absorption of amiben varied significantly between N sources. More amiben was absorbed in an NH_4^+ solution than was absorbed in NO_3^- solutions, and these responses interacted with solution pH. Higher pH values caused highly significant decreases in amiben absorption by corn and pigweed roots and corn coleoptiles. Amiben uptake decreased linearly as pH values increased from 4.0 to 8.0.

Bioassay experiments confirmed amiben was more phytotoxic to oats grown with either ammonium or nitrate sources of N, while solution pH had no effect on amiben phytotoxocity. Therefore, while amiben absorption may vary with N source and solution pH, these differences are not

reflected in amiben phytotoxicity. This indicates that the amiben and nitrogen interaction is not at the site of uptake.

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

INTRODUCTION

The first step in weed control by soil-applied herbicides is movement of the chemical from the soil into the plant. To date, little work has been done on the factors which control and influence herbicide uptake. However, there are many investigations and theories concerning inorganic ion uptake (as reviewed by Jennings, 1963; Sutcliffe, 1962; and Fried and Shapiro, 1961). In models proposed by these authors carriers are involved in transporting inorganic nutrients from the external solution, through the membrane wall, into the internal solution. It is not known if similar carrier systems exist for the active absorption of organic compounds. Competition for sites of uptake occurs between inorganic molecules of similar charge and size but such competitive effects between the nutrients and the organic molecules at the site of uptake have not been studied. Recently, workers (Dhillon, Byrnes and Merritt, 1967; Hogue, 1968; Nashed and Ilnicki, 1968) have studied the influence of herbicides on nutrient uptake and accumulation by plants, but little or no work has been done to study the influence of nutrients on herbicide uptake and phytotoxicity.

From a practical point of view, as the quantity of both the macro- and micro-nutrients applied to the soil continues to increase, it might become necessary to alter herbicide recommendations if herbicide phytotoxicity interacts with the nutrient status. One means of determining the effect of nutrients on phytotoxicity is to measure the quantity of herbicide absorbed. Herbicides and nutrients may interact at this site or within the plant.

The soil pH may also influence herbicide uptake and phytotoxicity by affecting the ability of the roots to absorb the chemical or by altering the character of the herbicide molecule. Knowledge of pH effects on the uptake of herbicides could help explain differential herbicide performance and crop tolerance in the field.

There is such a divergence of data and opinion on herbicide absorption that it is difficult to construct rational hypotheses concerning the effect of differences in the nutrient status on herbicide absorption and phytotoxicity. The objectives of this research were: (1) to study the influence of the nutrient level and combination on herbicide phytotoxicity, (2) to study root absorption of herbicides from various nutrient solutions, and (3) to study the effect of the hydrogen ion concentration on herbicide uptake.

LITERATURE REVIEW

The first report of herbicide uptake and nutrient interaction was by Crafts in 1939. He studied the effects of varying levels of Hoagland's nutrient solution on the toxic effects of borax, arsenate, and chlorate. Chlorate was less injurious to oats as the strength of the nutrient solution increased while borax and arsenate phytotoxicity were not affected. Further studies revealed that plants grown in a high level of nitrate absorbed little chlorate but cations apparently did not affect chlorate toxicity. This interaction is simply competitive uptake by two inorganic anions and under low nitrate levels the plant takes up more chlorate. This apparent competitive uptake was not reported by Fried and Shapiro (1961).

The early work which studied the interactions of organic herbicides and nutrients was with 2,4-dichlorophenoxyacetic acid (2,4-D) and its effects upon nutrient uptake and accumulation. Loustalot, Morris, Garcia and Pagan (1963) reported an increase in organic phosphorus in white bean (Phaseolus vulgaris L.) treated with 2,4-D. In contrast, Fang and Butts (1954) observed a significant decrease in the phosphorus content of treated white bean

leaves. Similar decreases were not present in the root and stem tissue. Supporting this work, Rebstock, Hamner and Sell (1954) also noted a decrease in the total phosphorus content in the leaves of 2,4-D treated P. vulgaris plants with no difference in the phosphorus content of the roots.

Wort (1962) noted the phosphorus content in tomato (Lycopersicon esculentum Mill.) was unaffected, increased in white beans, and decreased in soybeans (Glycine max (L.) Merr.) following an aerial application of 2,4-D. Thus the interaction effects of 2,4-D and the inorganic nutrients are complex and vary with species, concentration, time after treatment, and portion of the plant analyzed.

Alpha,alpha,alpha-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine (trifluralin) altered the region of phosphorus absorption by cotton and soybeans (Sabbe, 1967). Trifluralin placed in any of three soil zones, 0-5, 0-10, or 10-15 cm, shifted the zone of maximum phosphorus uptake. For example, cotton normally absorbs phosphorus in the 5-10 cm soil zone, but the application of trifluralin at 0.84 kg/ha in this region shifted the site of maximum uptake to the 0-5 and 10-15 cm zones. Further work by Oliver and Frans (1968) suggests that the decrease in phosphorus absorption in the treated region is due to the inhibition of lateral root development by trifluralin for both cotton and soybeans. Therefore, it is not a response due to a

herbicide and nutrient interaction in the soil or at the site of uptake but rather a morphological effect of the herbicide on root development.

Using P. vulgaris, Cooke (1957) measured the changes in concentration of potassium, chlorine, calcium, and sulfur at various times after 2,4-D applications. Potassium was found to increase after 8 hr but it had decreased after 24 hr. Chlorine followed the same trend at 12 and 48 hr after treatment, while the calcium content remained higher throughout the 48 hr period. The sulfur content increased slightly after 3 hr but was reduced after 24 hr. Cooke concludes these changes are secondary and probably are in response to the known increase in respiration which is then followed by a respiration decrease after a 2,4-D treatment.

One of the first reports of a nutrient and herbicide interaction by a chemical other than a phenoxyacetate compound was by Bingham and Upchurch (1959). Cotton and Italian ryegrass (Lolium multiflorum Lam.) were grown in solutions of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) in combination with several nutrient levels. They observed a highly significant interaction between phosphorus and diuron such that 2 ppm diuron reduced the fresh weight of cotton from 84% to 3% as the phosphorus level of a sandy loam soil increased. There was no similar interaction in a silty clay loam soil. For ryegrass the

interaction between phosphorus and diuron was highly significant in the sandy loam but not for the silty clay loam. Thus there is also an interaction between soil type, phosphorus level and herbicide. Soil nitrogen, potassium and pH levels had little influence on the effect of diuron on plant growth.

In a subsequent study, Upchurch, Ledbetter and Selman (1963) examined the relationship of soil phosphorus to the toxicity of twelve herbicides. No phosphorus and diuron interaction was observed. Of the twelve herbicides, only 3-amino-1,2,4-triazole (amitrole) interacted with phosphorus. At the zero P_2O_5 level, 8 ppm amitrole caused essentially no reduction in the dry weight of cotton in greenhouse conditions; whereas at the 160 mg P_2O_5/100 gm oven-dried soil level an 80% reduction in cotton growth was obtained from the same rate of amitrole. Similar interactions were observed in the field. Thus amitrole was apparently more phytotoxic at high phosphorus levels. The authors point out that possibly phosphorus was more phytotoxic in the presence of amitrole.

Vega (1964) also reported that there is an optimum phosphorus concentration for the enhancement of amitrole phytotoxicity. He found that phosphorus enhanced the uptake and/or translocation of amitrole-^{14}C and explained this by proposing that amitrole and phosphorus form a

complex. He does not state if the complex is within or external to the plant roots.

Ries, Larson and Kenworthy (1963) reported peach trees treated with 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine) and 3-amino-1,2,4-triazole plus ammonium thiocyanate (amitrole-T) had higher leaf nitrogen than where weeds were controlled by hand hoeing or plastic mulch. Simazine and amitrole-T influenced the nitrogen metabolism of apple trees also by increasing the total nitrogen. In 1965, Ries and Gast found simazine increased the nitrogen of corn (Zea mays L.), especially when grown at low nitrogen levels.

The source of nutrients also affects herbicide uptake and translocation. Recently, McReynolds and Tweedy (1969) observed that twice as much simazine-^{14}C was translocated to the shoots of corn, rye (Secale cereale L.), and soybeans grown in nitrate than in ammonium nitrogen.

An interaction between phosphorus and simazine has been reported by Adams (1964 and 1965). However, using eight soils, three plant species, three rates of simazine, and five phosphorus levels, only on two soil types was simazine significantly (10% level) more phytotoxic to soybeans. Adams concluded that the effect observed was a result of simazine decreasing the quantity of phosphorus required to produce salt toxicity to plants.

Dhillon, Byrnes and Merritt (1967) studied the effect of simazine on the uptake of ^{32}P by red pine seedlings

(Pinus resinosa Ait.). The results showed that simazine at levels of 5 and 10 ppm stimulated ^{32}P uptake, but inhibited uptake at higher levels of 15 and 20 ppm. In contrast to uptake, the rate of phosphorus translocation from roots to stems and needles appeared to be stimulated by all concentrations of simazine between 5 and 20 ppm. They postulate that the interaction of simazine and phosphorus at low concentrations may result in growth promotion while at high concentrations depression and injury. No determination of the simazine uptake at different phosphorus levels was made.

Recently the effect of 3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea (linuron) upon ion uptake by several species has been reported. Hogue (1968) applied sublethal and lethal doses of linuron to tomato and parsnip (Pastinaca sativa L.) foliage and determined its effect upon ^{32}P and ^{45}Ca uptake after 48 hr. Both levels of linuron stimulated ^{32}P uptake and translocation to the leaves in both species. The increase was in the inorganic phosphorus content. Linuron inhibited uptake and translocation of ^{45}Ca in both plants. Hogue concluded that the ^{45}Ca uptake inhibition and ^{32}P uptake stimulation point to different uptake mechanisms for anions and cations but no explanation for either effect is proposed.

The effect of linuron on ion uptake in corn, soybeans, and large crabgrass (Digitaria sanguinalis

(L.) Scop.) was investigated by Nashed and Ilnicki (1960). Linuron was applied in the nutrient solution and 7 days later the quantities of Ca, K, Mg, PO_4, NO_3, and SO_4 were determined. Linuron caused marked increases in the Ca and SO_4 content of the three species and also increased the NO_3 and PO_4 content of soybeans and the Mg content of crabgrass. The researchers noted that the increase in nutrient ions after the application of a photosynthesis inhibitor at lethal levels was unexpected. Substrates for all biochemical reactions including those involving active ion uptake would be reduced when photosynthesis was interrupted. They suggested that linuron affects the respiration which in turn caused an increase in ion absorption.

Rumburg, Engel, and Meggitt (1960) cite another example of competitive uptake between two inorganic anions. Phosphorus at high levels (62 ppm) completely prevented the expression of arsenate toxicity to oats, and the uptake of arsenate was 7.5 times greater at 1 ppm phosphorus in the nutrient solution than at 62 ppm. They concluded that phosphorus concentration was of major importance in relation to arsenate uptake and injury in grasses.

In 1964, Crafts stated that herbicides are taken up actively according to the scheme of Broyer (1956). In this scheme, a carrier system moved the substrate from the external solution to the internal cell solution. However,

Hartly, also in 1964, postulated that plants have not adapted to the uptake of herbicides, but that these "foreign" substances are expected to enter by passive diffusion. He argues that since many herbicides have a somewhat oil soluble form (e.g. 2,4-D acid but not the anion), they could penetrate into the suberized waxy tissues easier than dissolved salts could.

Donaldson (1967) measured and compared the absorption process of 2,4-D and monuron. Since 2,4-D required energy and was partially exchanged its uptake was concluded to be active. Monuron, on the other hand, could easily be exchanged after uptake and required no energy to be absorbed, indicating its uptake was passive.

Thus there are several different theories as to whether herbicide uptake is active or passive. Most workers studying herbicide uptake do not discuss their results in terms of the classical active and passive absorption mechanisms.

In a recent report, Pardee (1968) identified the "carriers" of nearly all transport models as membrane proteins. The processes of binding, translocation, and releasing the molecule being absorbed are all performed by proteins associated with or part of the cell membrane. He cited the active uptake by protein carriers of phosphoenolpyruvate, galactose, and β-galactosides. The molecular weights and sizes of such compounds are equivalent to those

of many herbicides. Thus it seems possible from the structural considerations of uptake that herbicides too could be actively accumulated by membrane protein.

In his recent book, Stein (1967) states that the flow of one substrate can stimulate flow of another in the opposite direction. An increase in the nutrient concentration of the culture solution increased the initial rate of nutrient uptake.

Recently, site of herbicide uptake studies have given valuable information concerning the mode of herbicide entry into plants. Knake, Appleby and Furtick (1967) reported that the shoot uptake of soil applied amiben by green foxtail (Setaria viridis (L.) Beauv.) was similar to the uptake of five other preemergence herbicides tested. Applications which caused a growth reduction of 50% for the tops of green foxtail caused no growth reduction when applied to the root zone. They conclude shoot uptake is the pathway which resulted in the lethal action of herbicides to green foxtail. The unpredictability of herbicide performance is illustrated by the fact that Knake and Wax (1968) discovered that amiben was absorbed differently by giant foxtail (Setaria faberii Herrm.) application of amiben at 1.5 ppm in the root zone reduced the dry weight of the top growth 50% while application to the shoot zone reduced it 27%. This response was different than that of the other herbicides. Placement of ten other chemicals in the shoot

zone proved more phytotoxic than placement of the same concentration in the root zone.

A factor that has pronounced effects on nutrient ion availability and adsorption in the soil is the pH of the soil or growing media. Not much is known about the soil pH effects on herbicide phytotoxicity. In a synthetic soil media, Weber, et al. (1968) observed that the weakly basic 2,4-bis(isopropylamino)-6-methylthio-s-triazine (prometryne) was more phytotoxic to wheat (Triticum aestivum L. em. Thell.) as pH increased. They concluded that the reduction in phytotoxicity at the lower pH was due to the adsorption of protonated prometryne by the clay colloid which reduced its concentration in the solution phase of the growing media.

Corbin and Upchurch (1967) studied the influence of pH on the detoxification of soil applied herbicides and found highly significant effects. They state that not only can performance of soil-applied herbicides be easily influenced by the regulation of the detoxification rate by soil pH, but that this is only one way in which pH can influence field performance.

In an inclusive study of many soil and climatic factors which affect herbicidal activity, Upchurch, Selman, Mason and Kamprath (1966) correlated the soil pH to herbicide performance. There was no or poor correlation of pH effects on the performance of both N,N-diallyl-2-chloro-

acetamide (CDAA) and 2-chlorallyl diethyldithiocarbamate (CDEC) at 17 locations for 3 years. Phytotoxicity of simazine, diuron, and isopropyl N-(3-chlorophenyl)carbamate (CIPC) to cotton and soybeans was generally negatively correlated to soil pH. For crabgrass there was no correlation between phytotoxicity and soil pH.

Contrary to these findings, Nash (1968) measured a significant increase in the amount of diuron in oat shoots grown for two weeks in sandy loam as the soil pH increased. The roots had equal quantities of diuron-^{14}C in oats grown in soils with pH's 4.7, 6.4 and 7.9. The pH changes had no measurable effects on oat shoot growth while root growth increased as pH increased.

Rains, Schmid and Epstein (1964) theorized that a low pH of the external solution might affect the ion carriers of the root such that the rate of cation absorption would be reduced due to competition between hydrogen ions and the substrate cation for available carrier sites. Similarly, at high pH values hydroxyl or bicarbonate ions might compete with substrate anions, thereby reducing the rate of anion absorption. They also discuss possible damage by hydrogen ions to the ion absorption mechanisms. By measuring rubidium-86 (^{86}Rb) absorption in a 10 min period, they found competition with the hydrogen ions for carrier sites. At pH 3.9 the rate of ^{86}Rb absorption decreased with time in the absence of calcium, whereas with 0.50 mM

Ca it remained constant. Further study revealed that calcium was completely able to prevent hydrogen ion injury to the carrier mechanism.

In 1958, Fried and Noggle observed that hydrogen ions not only compete for rubidium carrier sites but also for potassium, sodium and strontium sites. They further noted that when the cation concentration was appreciably higher than the hydrogen ion concentration there was little or no pH effect due to competition. However when the ion concentrations were similar, marked pH effects occurred. No hydrogen ion injury was shown.

MATERIALS AND METHODS

Preliminary experiments were performed to establish herbicide concentrations which would reduce the top growth of oats by 50% (hereafter called the GR_{50} value). The effects of the absence of nitrogen (N), phosphorus (P), and potassium (K) when compared to a complete nutrient solution on plant growth in the presence of a herbicide were also determined.

Oats (Avena sativa L.) 'Clintland 64' were grown for 3 weeks in sand culture in the greenhouse as a bioassay plant in different nutrient and herbicide solutions. Fifteen seeds were planted 1 cm deep in nonacid washed # 7 graded quartz sand in a 6 oz styrofoam cup with drainage holes punched in the bottom. This cup was placed inside a 10 oz waxed cold cup which contained 120 ml of the treatment solution. In this manner the oats in the styrofoam cup were subirrigated by the solution in the waxed cup. The oats were thinned to 10 plants per cup after emergence. The daily addition of water maintained the solutions at their original volume but not at the initial nutrient level. The greenhouse temperature was approximately 23 C and the day-length was extended to 14 hr with overhead

flourescent lights. The four nutrient treatments were complete Hoagland's # 1 solution, Hoagland's minus N, Hoagland's minus P, and Hoagland's minus K. All solutions contained the other essential elements and were adjusted to pH 6.0 with 0.1 N NaOH. Herbicide treatments were 0, 4, 8, or 16 ppm (w/v) formulated atrazine or amiben in combination with the nutrient solutions. The treatments were replicated three times in a completely randomized design. Visual injury symptoms on a 0 to 10 scale (0 = no injury, 10 = complete kill) and dry weights of the tops were taken 3 weeks after planting. The effect of the absence of one element from the solution was determined by dividing the dry weight of the 10 oat shoots by that produced by the same nutrient treatment without the herbicide.

A factorial experiment with three levels of N, P, K and a herbicide was designed to study further the results of the preliminary test. Clintland 64 oats were seeded and grown in the manner described previously. Treatments were three levels of N, P, and K (0, one-half, and full-strength Hoagland's) in all combinations with either 0, 4, or 8 ppm (w/v) amiben or 0, 1 or 2 ppm (w/v) atrazine or linuron. This 3^4 factorial gives 81 treatment combinations for each herbicide and the experimental design was a randomized block with two replications. Plant injury ratings, dry weights of the tops, and solution pH values were determined after 3 weeks growth.

Previous bioassay experiments were performed with a nitrate source of nitrogen (NO_3^-). In a final study the phytotoxicity of a herbicide with an ammonium source of nitrogen (NH_4^+) was compared to the phytotoxicity in NO_3^- solutions. Simultaneously, the effect of the nutrient solution pH on herbicide phytotoxicity was measured. In a factorial arrangement five pH values (4.0 to 8.0) were combined with nitrogen levels of 0, 10 and 50 mM NO_3^- or NH_4^+ in two replications in a randomized complete block design.

To inhibit bacterial growth in the solutions, 20 ppm (w/v) streptomycin sulfate were added to all treatment solutions. The conversion of nitrate to ammonium in the NO_3^- solutions was prevented by adding N-serve at the rate of 0.1 ml/mM NO_3^- per liter of solution. Amiben was the only herbicide tested and the rates were 0 and 8 ppm. Fifteen oat seeds were planted as in the previous bioassay experiment. These were thinned to 10 plants per cup after emergence. Water, adjusted to the proper pH, was added daily to bring the solutions to their original volumes. Injury ratings and dry weights of the shoots were taken after 3 weeks growth. Solution pH values were also taken at the termination of the study. Planned comparisons were made on the injury ratings and the percent growth reduction of the shoots caused by amiben at the different nitrogen and pH levels.

I. Herbicide Absorption by Roots

To determine if the phytotoxicity differences in various nutrient solutions were due to nutrient effects on herbicide entry into the root, the effects of mineral nutrition on herbicide absorption were investigated. By using radioactive herbicides, the quantity of herbicide entering a plant at nontoxic concentrations was measured. This made possible the determination of nutrient influence on herbicide uptake separate from the physiological effects of the herbicide which could affect subsequent herbicide absorption (Crafts, 1959). The system did not measure an interaction between a nutrient and the herbicide on phytotoxicity within the plant since the treatment period was short.

A preliminary trial indicated that a treatment period of 4 hr to 5-day old corn, 7-day old soybeans, and 14-day old pigweed plants in nutrient solutions containing 0.5μc radioactivity per 100 ml nutrient solution gave sufficient radioactivity in the roots for accurate detection.

Roots of 'Harosoy 63' soybeans, 'WF9 X OH51A' single cross corn, and redroot pigweed (Amaranthus retroflexus L.) were treated with ^{14}C-labeled herbicides in various nutrient combinations and levels. Prior to treatment, the plants were grown in quartz sand with complete Hoagland's # 1 nutrient solution. They were germinated

under 21/27 C night/day temperatures with 35% daytime relative humidity under a 14 hr photoperiod of 2950 ft-c. The plants were removed from the sand as carefully as possible but some root damage did occur, especially with corn. The roots were rinsed three times in distilled water, blotted on tissue to remove excess water, and then placed in 70 X 21 mm shell vials containing 10 ml of the treatment solution.

The treatments were 0, 10 and 50 mM NO_3^-; 0, 1 and 5 mM PO_4^{3-}; and 0, 5 and 25 mM K^+ which are 0, 1X and 5X the quantities of each nutrient recommended in Hoagland's # 1 solution, respectively. Nitrogen was supplied as KNO_3 and $Ca(NO_3)_2$, phosphorus as $Ca(H_2PO_4)_2$, and potassium as K_2SO_4. The solutions were complete for all other elements, including the micronutrients, so that there would be no serious nutrient imbalances which might affect selective ion absorption (Epstein, 1961; Elzam and Epstein, 1965). Solution pH was brought to 6.0 with 0.1 N NaOH.

The radioactive carbon position and specific activity for the herbicides were as follows: carboxy-labeled amiben-^{14}C, 2.19 mc/mM; uniformly ring-labeled atrazine-^{14}C, 1.32 mc/mM; and carbonyl-labeled linuron-^{14}C, 1.70 mc/mM.

Two herbicide concentrations were used, one which was nontoxic and another which would cause a growth reduction of 50% to susceptible plants grown 3 weeks with the herbicide. Tne nontoxic concentrations were prepared with

the undiluted labeled materials to give .5μc radioactivity/ 100 ml nutrient solution. Thus the molar concentration is dependent upon the specific activity of the labeled compounds. The nontoxic concentrations were 2.3 X 10^{-6} M amiben, 3.8 X 10^{-6} M atrazine, and 2.9 X 10^{-6} M linuron and the GR_{50} concentration was 10 X 10^{-6} M for all three herbicides. Nonlabeled technical material was added to the labeled materials to prepare the 10 X 10^{-6} M solutions.

After the 4 hr treatment period, the plants were removed from the vials, the roots rinsed 3 times in distilled water, blotted dry on tissue, cut from the plant, and wrapped in preweighed sample wrappers which were subsequently used in the Schoniger oxygen combustion technique for carbon-14 analysis. The roots were dried at 60 C for 24 hr and weighed. The entire root system from each plant was analyzed.

To determine if any herbicide-^{14}C molecules were adsorbed only to the outside of the root and were not removed by rinsing, the roots of several plants of each species were immersed in a treatment solution of each herbicide. After 5 - 10 sec the roots were removed and washed as described above. Analysis confirmed that essentially no herbicide was adsorbed to the outside of the root that was not removed by the triple rinsing technique.

The analysis technique was basically the Schoniger combustion method of Wang and Willis (1965). The paper

containing a plant sample was placed in a nichrome wire basket attached to a 14 cm length of nichrome wire. The wire was inserted into a # 8 rubber stopper and the stopper and basket were placed in a one-liter suction flask and a vacuum drawn. The flask was then filled with oxygen and evacuated three times. It was then clamped off, placed in a Thomas-Ogg infrared combustion chamber, and ignited. The evolved $^{14}CO_2$ was absorbed by 20 ml ethanolamine-ethanol solution, 1:2 (v/v). The trapping solution was injected into the flask through a serum vial cap in a 4 X 80 mm glass tube in the rubber stopper. Five milliliters of the solution were removed 30 min later and placed in a scintillation counting vial with 10 ml scintillation solution containing 5.0 g 2,5-diphenyloxazole (PPO) and 0.3 g 2-p-phenylene-bis(5-phenyloxazole) (POPOP) in 1 liter toluene. The vials were placed in a Packard tri-carb liquid scintillation spectrometer and 10 min counts taken. Color and chemical quenching were determined using internal toluene-^{14}C standards and the channels ratio method (Hergerg, 1965). The counting efficiency was consistently between 71.1 and 72.8%.

The counts per minute were converted to disintegrations per minute per mg plant root tissue (dpm/mg). Combustion efficiency was determined by spotting known quantities of the labeled herbicides directly on sample wrappers. The percent recovery for each herbicide was

consistently 92-95%. Since this is quite high, no correction was made for the missing 5-8% radioactivity. Analysis of variance was performed on the data and planned comparisons made to determine the treatment effects.

Experiments were designed to study the effects of nitrogen source and solution pH on the absorption of amiben by corn and pigweed roots. Two levels of nitrate (NO_3^-) and ammonium (NH_4^+), 10 and 50 mM, and a solution with no nitrogen were mixed in all combinations with five pH values, 4.0, 5.0, 6.0, 7.0 and 8.0. Solutions were mixed and the pH's adjusted just prior to treatment to reduce the possibility of microbial effects upon the nitrogen level and pH of the solutions. The factorial design was completely randomized in two replications. The treatment time was 4 hr and the amiben concentration was 2.3×10^{-6} M with a radioactivity of 0.5 μc/100 ml solution. The corn and pigweed plants were treated and the roots analyzed as described previously. The results were analyzed and orthogonal comparisons were made.

II. Amiben Absorption by Corn Coleoptiles

An experiment was initiated to determine if amiben absorption by corn shoots was similar to root absorption in solutions of different nitrogen sources and levels and different pH values. In order to use intact coleoptiles,

a technique was developed whereby only the unbroken coleoptiles of corn were treated. Three corn seeds, 'WF9 X OH51A', were placed on 2 cm of quartz sand in 6 oz styrofoam cups. A hole was punched in the side of each cup 2 cm from the bottom so excess water could drain. The cups were then filled with vermiculite, watered with complete Hoagland's solution and placed in a dark controlled environment chamber for 3 days with 12 hr 27 C and 12 hr 22 C. The chamber was kept dark to simulate underground growing conditions so that the true leaves would not penetrate the coleoptile. After 3 days the coleoptiles were 25-35 mm long and were suitable for treatment.

The cups were cut down the side vertically to 2 cm from the bottom and then cut completely around at this height. The top portion of the cup was discarded and the vermiculite was carefully removed around the coleoptiles. The two most uniform coleoptiles were selected for treatment. A moderate quantity of "stainless putty" (Sure Seal Products Co., Chicago 22, Ill.) was applied around the base of each coleoptile to form a water tight seal. A 5 cm section of plastic drinking straw was then placed over the coleoptile and into the putty base. The treatment solution was pipetted into the straw until the liquid was approximately 10 mm above the coleoptile tip. The treatments were 0, 10, and 50 mM of either NO_3^- or NH_4^+ in all combinations with pH's 4.0 to 8.0 as in the previous root

absorption experiment. The level of the solution was marked on the straw so that leaks could be detected. The cups were again placed in the dark chamber at 27 C. After 90 min the straws were removed. A preliminary experiment indicated that during longer treatment periods leaks developed around the coleoptile base. The coleoptiles were cut at the base of the treated area. They were rinsed three times in distilled water and blotted dry. Their length was recorded and they were then placed in preweighed sample wrappers. Analysis for the radioactive amiben was performed as it was with the root tissue.

This technique provided uniform plant coleoptiles and there were no problems treating intact coleoptiles for relatively short periods. One disadvantage of the system was that only a small quantity of treatment solution could be applied. This could be a problem if absorption was very rapid and the concentration of the external solution changed to significantly lower levels. This was not a problem with amiben in these experiments since the rate of uptake was relatively slow.

RESULTS AND DISCUSSION

Concentrations of 4 and 8 ppm amiben were necessary to obtain a GR_{50} value while all levels of atrazine were well above this value (Figures I and II). Based on this information, 4 and 8 ppm amiben and 1 and 2 ppm atrazine were used in the following experiment.

The results were analyzed by planned comparisons so that specific effects could be observed. The main effects were the herbicide rates and the nutrient combinations, and the remaining degrees of freedom for treatments are the interaction effects (Tables I and II, Appendix).

For amiben, a highly significant linear relationship was observed between concentration and percent reduction in dry weight of the oat shoots. Only the absence of N in the nutrient solution caused a significantly different response than the complete nutrient solution. With no N, the phytotoxicity of the amiben was greatly reduced.

There was a significant interaction with the linear effects of amiben and the NPK vs -N comparison. Comparing the percent dry weight reductions for the NPK nutrient solution at all three herbicide levels with the values for -N shows that the reduction is much less at 4 ppm amiben than at 8 and 16 ppm.

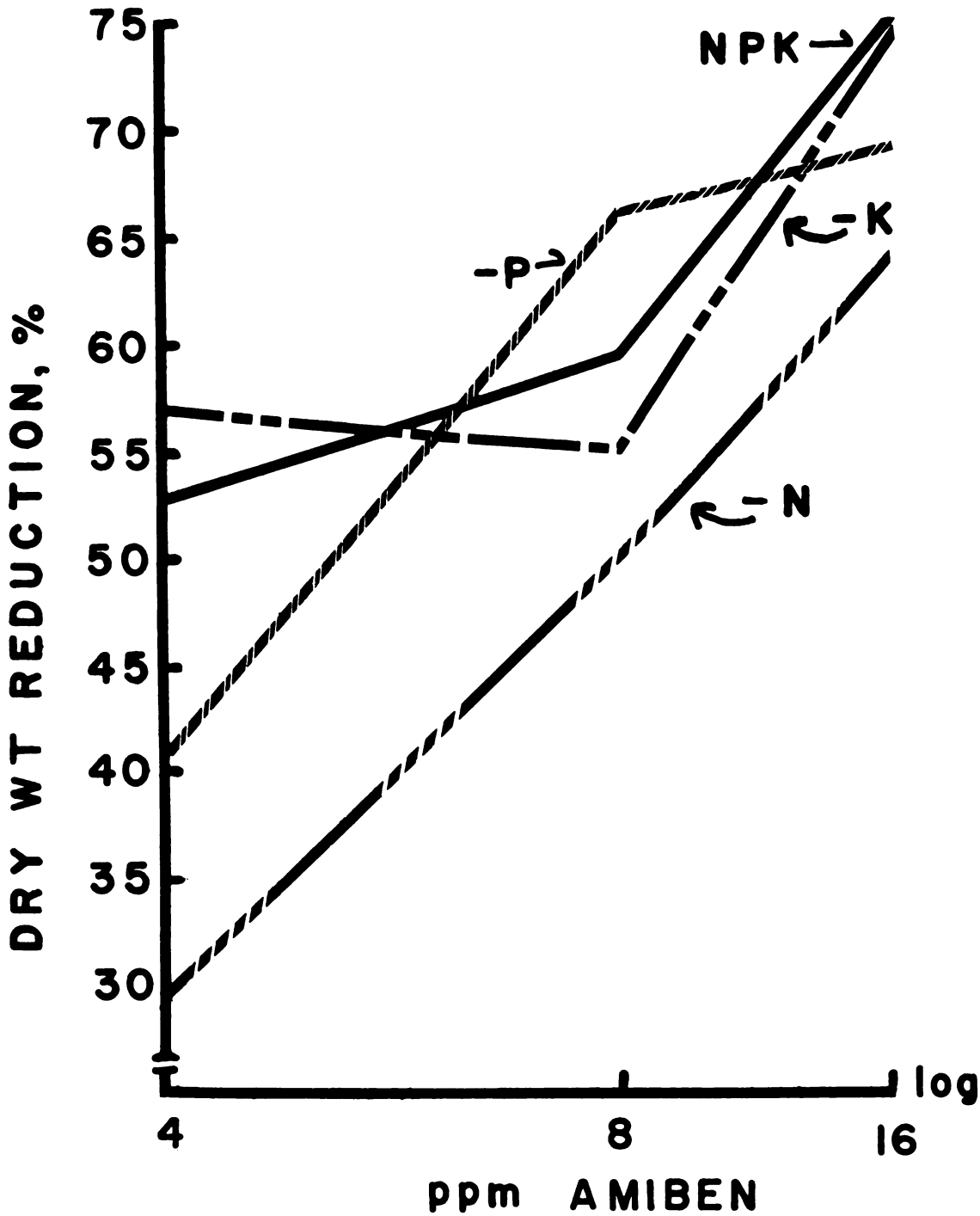

Figure I. Phytotoxicity of amiben to oats grown in sand culture 3 weeks in complete and nutrient deficient solutions

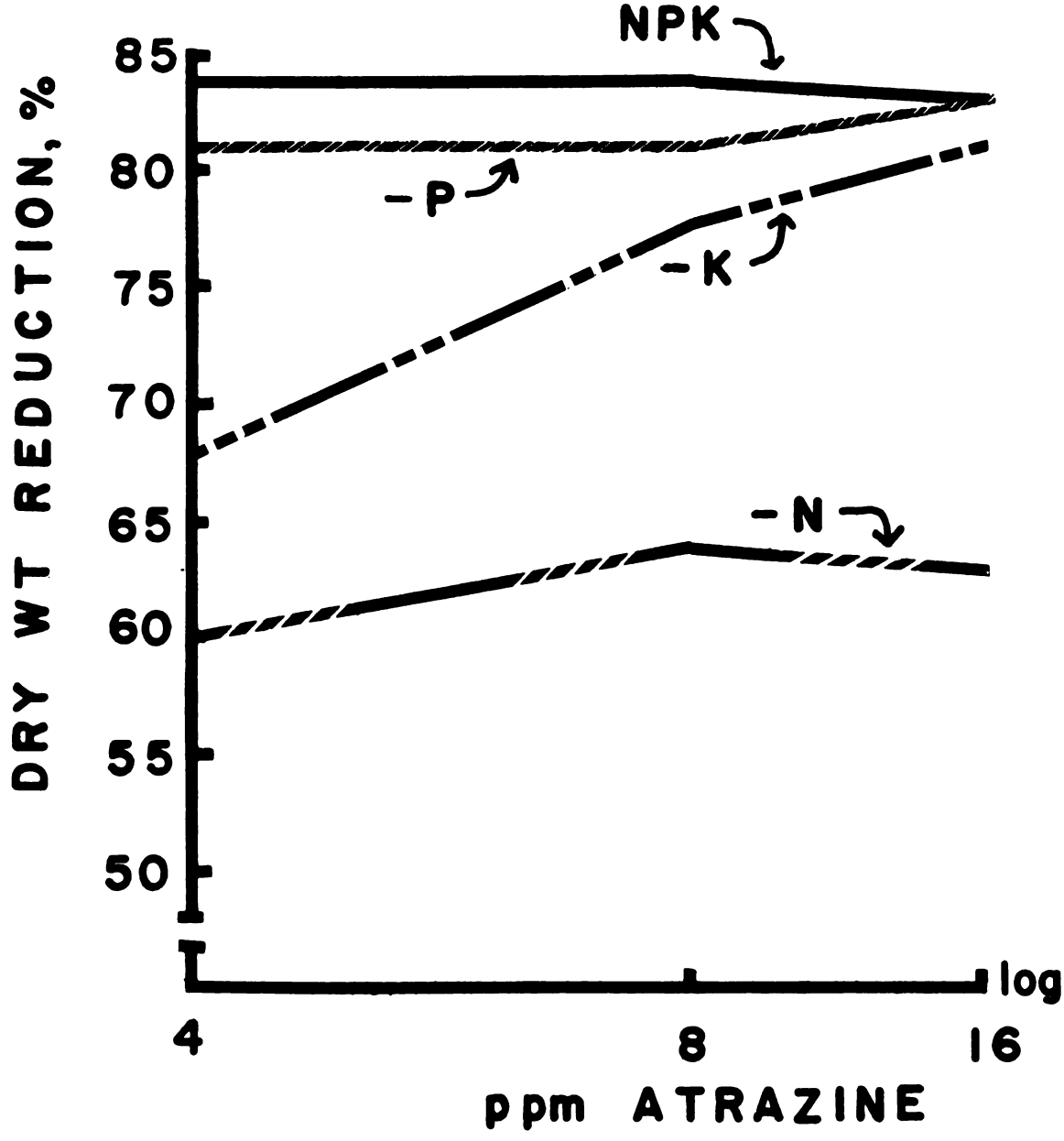

Figure II. Phytotoxicity of atrazine to oats grown in sand culture 3 weeks in complete and nutrient deficient solutions

There is a highly significant quadratic X NPK vs -P interaction due to the reversal of the phosphorus effect at 8 ppm of amiben. Amiben at 4 and 16 ppm was less phytotoxic with no P than with NPK, but at 8 ppm it was more phytotoxic with no P. No explanation is proposed for this interaction as it was not observed in subsequent experiments.

The levels of atrazine were all too high to give a GR_{50}, and yet there was a significant linear effect (Table II, Appendix). There were highly significant differences between the complete solution and the lack of either N or K. The absence of P had no effect upon the toxicity to oats.

The absence of N (Figure II) greatly reduced the phytotoxicity of atrazine. Dry weight reductions in the oat shoots with K absent at 4 ppm atrazine were significantly lower than at 8 and 16 ppm. No explanation of this latter interaction is proposed.

Therefore, the important effect is that no N in the nutrient solution greatly reduces amiben and atrazine phytotoxicity to oats grown in sand culture.

A factorial experiment with three levels of N, P, K and amiben, atrazine, and linuron was designed to determine any interactions between the nutrients and herbicide phytotoxicity. For all herbicides, the only meaningful interactions were with N (Tables III, IV, and V, Appendix).

The interaction effect was the same: the phytotoxicity increased as the N level increased. There were no interactions with either P or K as observed in the first experiment with amiben and atrazine.

As expected, increasing the amiben rate increased the injury ratings. As the N level increased, dry weights also increased. Even though the main effect of increasing N on injury ratings appeared to decrease the injury, interpretation of the N and amiben interaction (Figure IIIa) revealed a different effect on injury ratings. The symptoms of N deficiency in plants growing with zero N were not distinguishable from the symptoms of amiben injury. This accounts for an apparent injury rating of 2.0 for the zero N and zero amiben treatment. If we assume that the plants growing in 4 and 8 ppm amiben and zero N express the same degree of N deficiency symptoms plus the herbicide damage, the 0 mM NO_3^- line can be corrected to show only the herbicide injury and not the deficiency symptoms by subtracting 2.0 from the injury rating at all three amiben levels. This brings the zero level N injury ratings at all three amiben levels below the injury ratings for 5 and 10 mM NO_3^-. There were no observable N deficiency symptoms at either 5 or 10 mM NO_3^- and no adjustment is required for these treatments.

That amiben was more toxic as the N level increased can also be supported by comparing the slopes of the three lines in Figure IIIa. The degree of plant injury increased

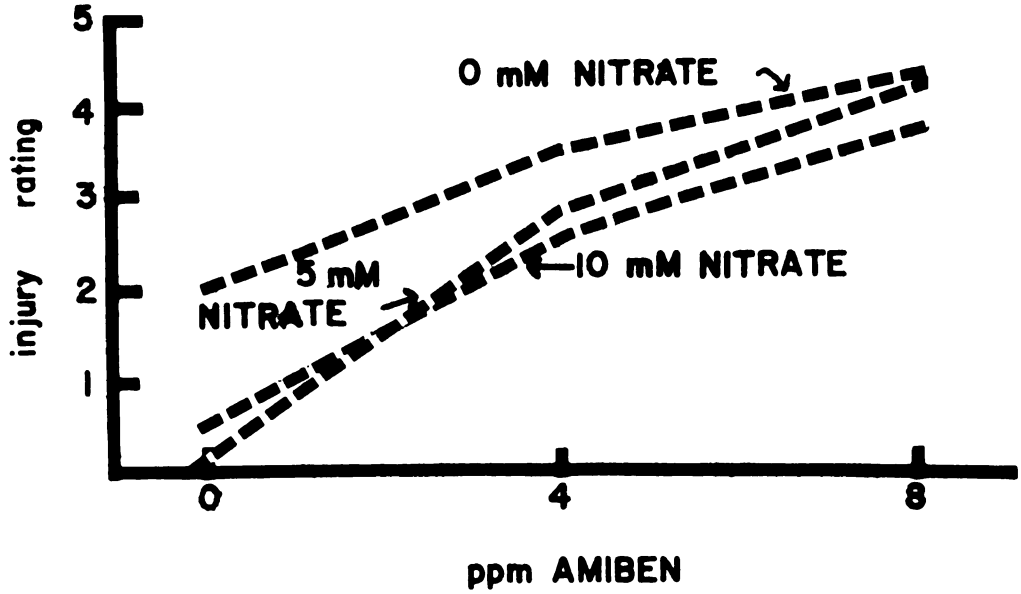

Figure IIIa. Interactions of amiben and nitrate on the injury of oat shoots grown in 3 weeks in sand culture (0=no injury; 10=complete kill)

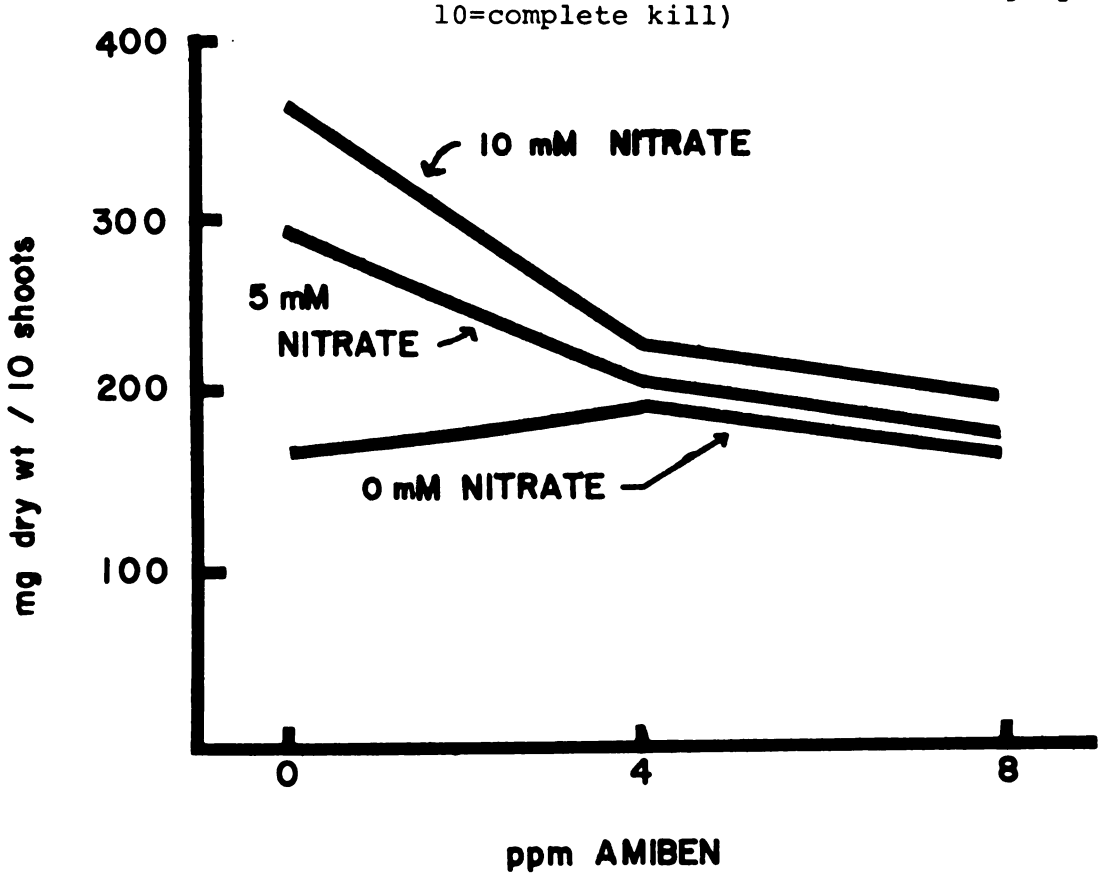

Figure IIIb. Interactions of amiben and nitrate on the dry weight of oat shoots grown 3 weeks in sand culture

more rapidly (i.e. the slope of the lines is greater) for the 5 and 10 mM NO_3^- lines than it did for the 0 mM NO_3^- line. As amiben was increased from 0 to 4 and 8 ppm, the absolute value of the difference in injury ratings between the two amiben rates for a given N level was greater for 5 and 10 mM NO_3^- than for no N. With no N, there was a slight dry weight stimulation as the amiben increased from 0 to 4 ppm. Even 8 ppm amiben did not reduce shoot growth with zero N. However, with N present, amiben was significantly more phytotoxic and the dry weight reduction was even greater with 10 mM NO_3^- than with 5 mM.

The main effects of atrazine on the injury ratings and dry weight of the oats were the same as those for amiben (Table IV, Appendix). Injury ratings were different between all three atrazine rates. There was a reduction in the dry weight of oats between the 0 and 1 ppm rates with no differences between 1 and 2 ppm. As the N level increased, the dry weight increased at all three levels and the injury rating increased also. This statement appears contradictory in that both injury and dry weights increased as the NO_3-N level increased.

There were highly significant differences in the injury ratings at 1 and 2 ppm atrazine between the 0 mM NO_3^- level and the 5 and 10 mM levels (Figures IVa and IVb). There were no significant differences between 5 and 10 mM NO_3^- on plant injury. The significant interaction effects

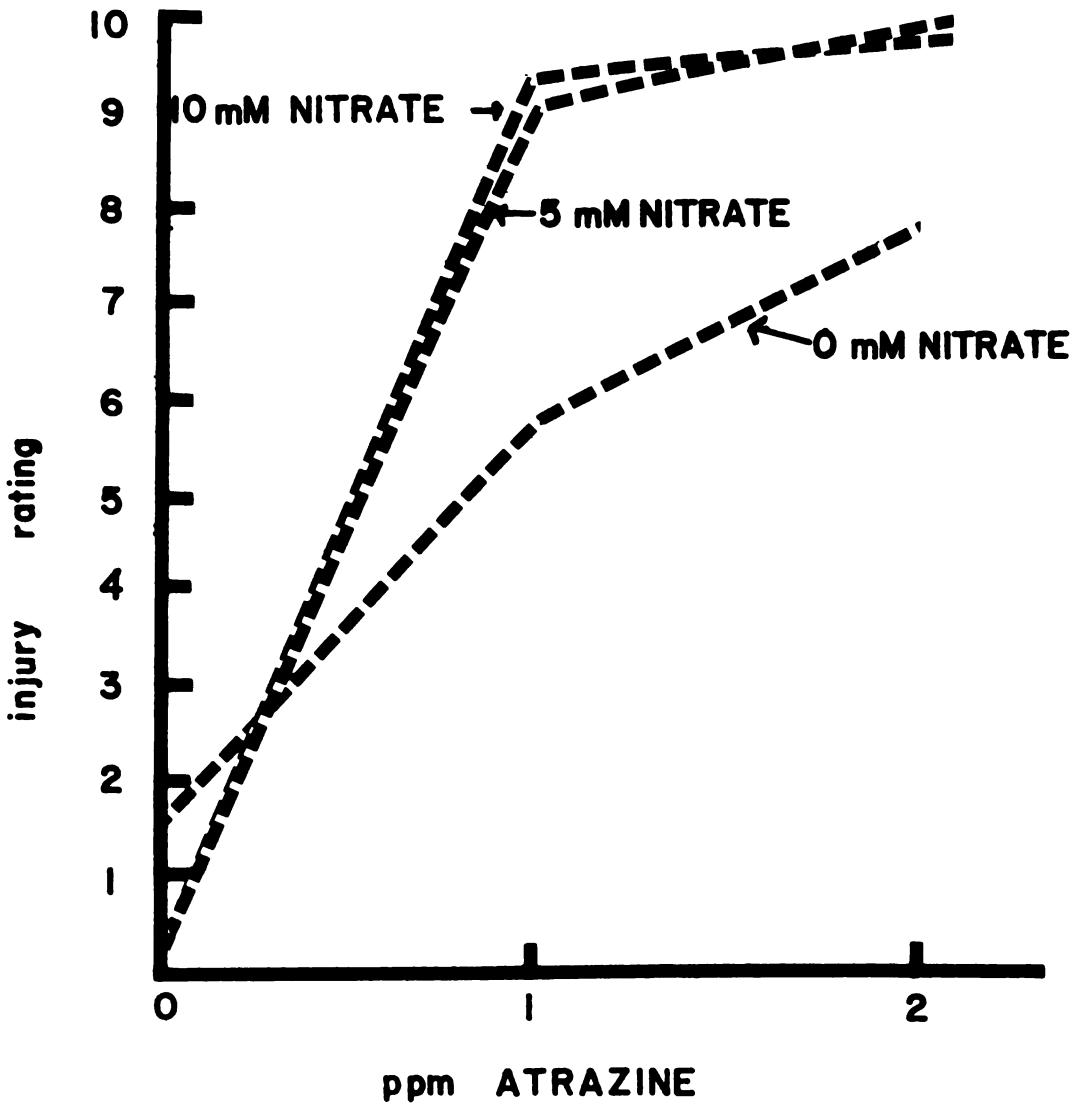

Figure IVa. Interactions of atrazine and nitrate on the injury of oat shoots grown 3 weeks in sand culture (0=no injury; 10=complete kill)

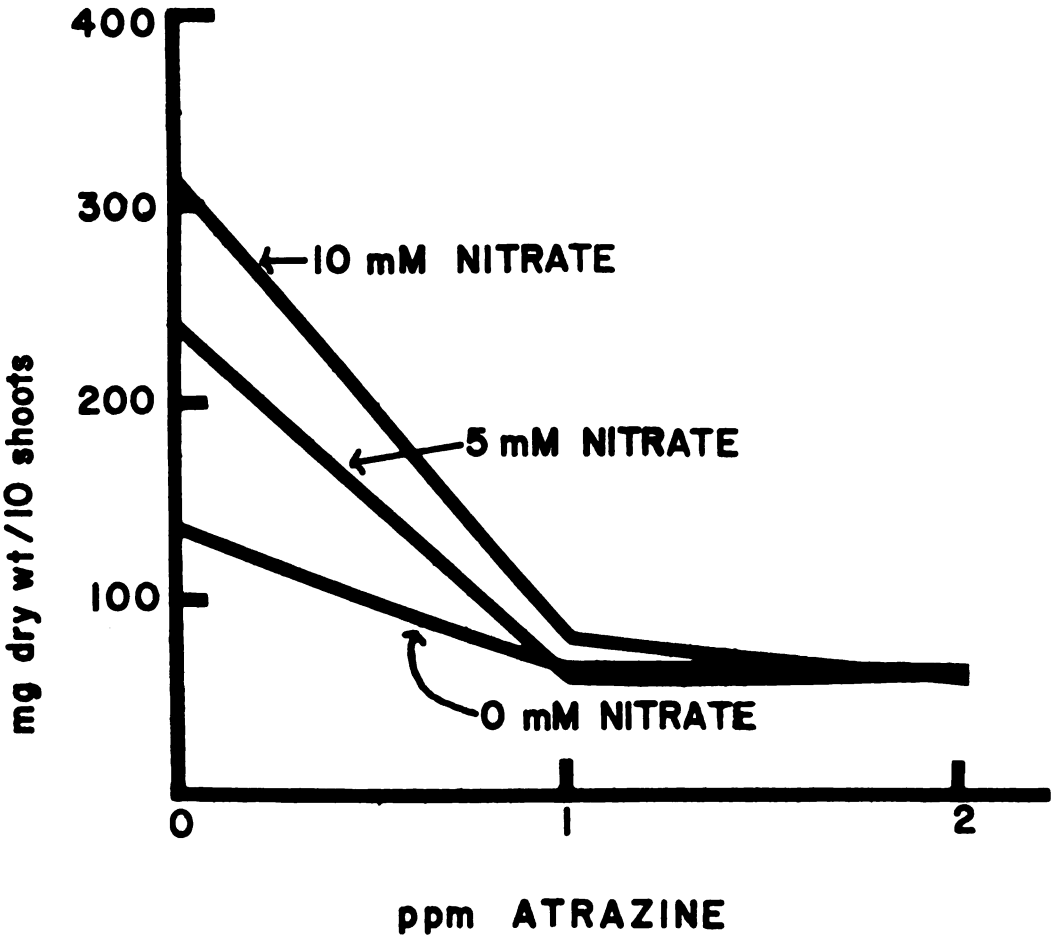

Figure IVb. Interactions of atrazine and nitrate on the dry weight of oat shoots grown 3 weeks in sand culture

on dry weight (Figure IVb) shows there was much greater reduction in weight when N was present. The interaction between the 5 mM and 10 mM NO_3^- and 0 vs 1 and 2 ppm atrazine was also highly significant.

Two points are of particular interest. Without N, atrazine reduced the oat growth relatively little. These oats were obviously affected by the absence of N and this deficiency prevented as great an expression of atrazine phytotoxicity. The triazines inhibit the Hill reaction of photosynthesis (Moreland, Gentner, Hilton and Hill, 1959). When N was limiting the importance of photosynthesis was probably reduced since the rate of vegetative growth was less and thus the atrazine appeared less phytotoxic than when N was abundant.

The other point is that for all three N rates, the growth of the oats was reduced the same extent when atrazine was present. It seems possible that the lines for the 5 and 10 mM NO_3^- rates could have been parallel (Figure IVb) but atrazine at all three rates prevented the oats from producing more than a given dry weight at all N levels.

The question of how N caused greater plant injury and also increased plant weight still remains. It is known that N stimulates vegetative growth which is dependent upon a functional photosynthetic mechanism. Thus plants supplied with high N levels initially grew more rapidly and increased in weight over those plants at the zero N level.

These same plants also became dependent upon assimilated materials sooner than the others; and when atrazine was present, these plants were not able to supply the products normally available from photosynthesis. Thus the symptoms of atrazine phytotoxicity appeared sooner in the life cycle of plants supplied with N than in those without it. The injury ratings therefore increased as the N rate increased since the higher N levels caused the plants to become dependent upon photosynthesis earlier.

The response of oats to linuron under different N levels as determined by injury ratings and dry weights was identical to that of atrazine. The main effects of linuron and N show injury increased significantly for all three linuron and N rates. Linuron greatly reduced the dry weight of oat shoots, but there was no difference in the reduction between the 1 and 2 ppm rates. The presence of N significantly increased the dry weight above 0 mM NO_3^-, but there was no difference in weight between 5 and 10 mM NO_3^-.

As with atrazine and amiben, only the N and linuron interactions were significant. Figure Va shows that linuron was significantly more phytotoxic with than without N and that there was also more injury at 10 mM NO_3^- than at 5 mM. The dry weight reductions (Figure Vb) increased as the N levels increased and they were reduced to the same value by both 1 and 2 ppm linuron.

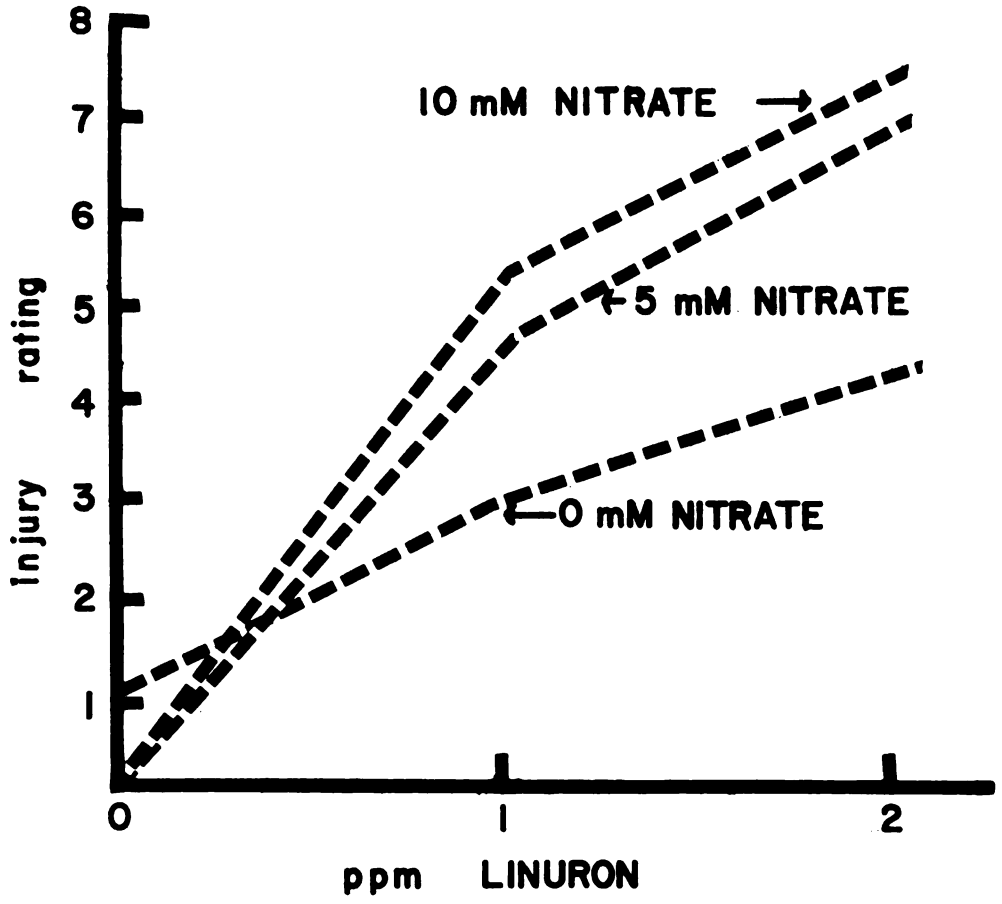

Figure Va. Interactions of linuron and nitrate on the injury of oat shoots grown 3 weeks in sand culture (0=no injury; 10=complete kill)

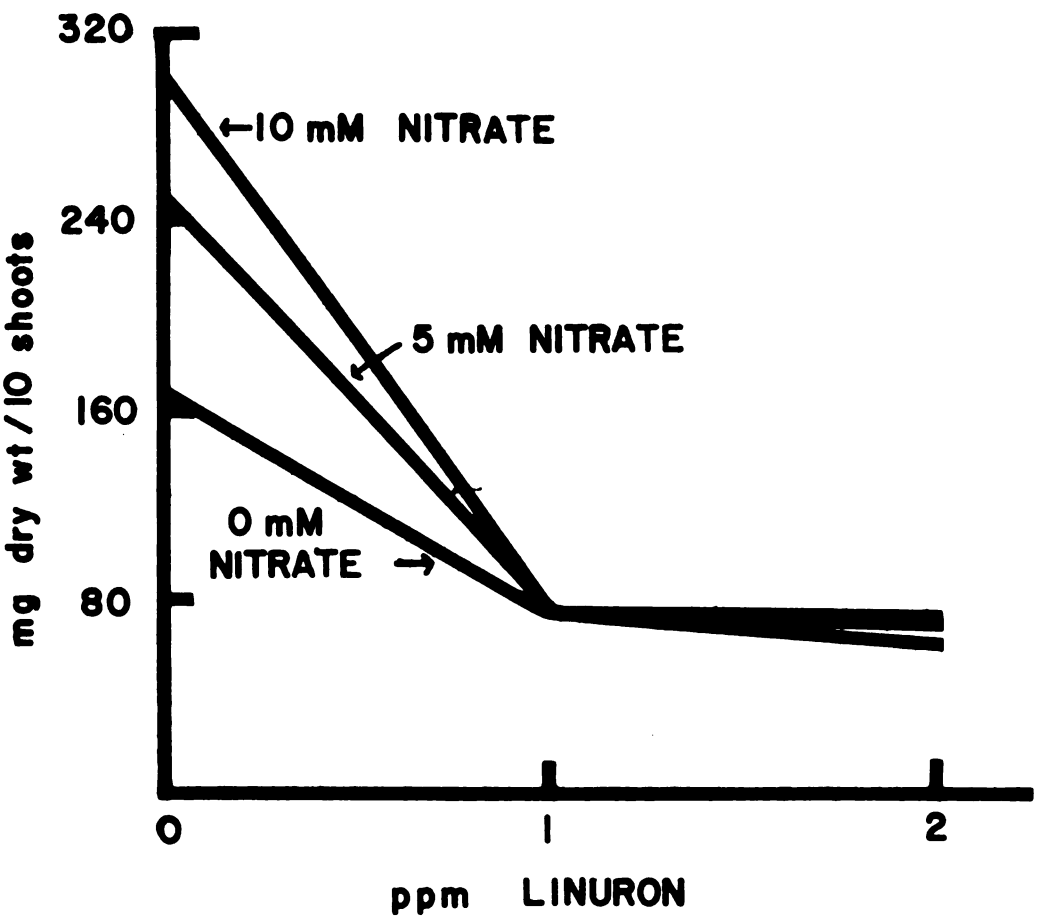

Figure Vb. Interactions of linuron and nitrate on the dry weight of oat shoots grown 3 weeks in sand culture

There were no interactions between the three N and the 1 and 2 ppm linuron levels.

The fact that the linuron interactions with N are identical to those of atrazine is not surprising since linuron too is an inhibitor of the Hill reaction (Cooke, 1956). Therefore the explanation of why both the injury rating and dry weights of oats increased as the N level increased is the same as the previous discussion for atrazine.

No P or K interactions on phytotoxicity were found. It was not possible to isolate the mechanism of the N interaction in bioassay studies of this type. Either the increased susceptibility to herbicides was due to an interaction of the herbicide and N within the plant or the N level of the solution influenced the amount of herbicide entering a plant. The herbicide absorption experiments were designed to determine nutrient status effects on herbicide entry into plants.

The experiments which measured herbicide absorption by roots showed that amiben uptake (Table 1) in both corn and pigweed decreased significantly with the addition of NO_3^-. There were no significant differences between the 10 and 50 mM NO_3^- rates.

The addition of NO_3^- had no effect on atrazine uptake (Table 2) by corn or pigweed. There was a significant

Table 1a. Amiben-^{14}C absorption by corn, soybeans, and pigweed roots in 4 hr as influenced by nitrogen, phosphorus, and potassium levels in the nutrient solution

Amiben absorption, dpm/mg root, dry wt									
	Corn			Soybeans			Pigweed		
Amiben concn X 10^{-6} M	2.3	10	avg	2.3	10	avg	2.3	10	avg
Nitrate									
0	366	355	360	624	783	704	70	76	73
10 mM	257	204	230	616	596	606	58	53	56
50 mM	232	240	236	673	624	648	62	51	56
avg	285	266	276	638	668	653	63	60	62
Phosphate									
0	282	283	282	867	705	786	82	64	73
1 mM	310	287	298	700	530	615	58	53	56
5 mM	305	282	294	838	574	706	80	66	73
avg	299	284	292	802	603	702	73	61	67
Potassium									
0	269	185	227	648	616	632	53	60	56
5 mM	264	229	246	745	569	657	58	53	56
25 mM	262	251	256	620	545	582	52	54	53
avg	265	222	244	671	577	624	54	56	55
avg for N,P,K	283	257	271	704	616	660	63	59	61

Table 1b. Planned comparisons of amiben and nutrient effects and their interactions on amiben-^{14}C absorption by corn, soybeans and pigweed roots in nutrient solutions

Comparison	Significance		
	Corn	Soybeans	Pigweed
Amiben rate effect			
2.3 X 10^{-6} M (H_1) vs 10 X 10^{-6} M (H_2) amiben	NS	**	NS
N,P,K main effects			
0 vs 10 and 50 mM nitrate	**	NS	**
10 vs 50 mM nitrate	NS	NS	NS
0 vs 1 and 5 mM phosphate	NS	**	NS
1 vs 5 mM phosphate	NS	*	**
0 vs 5 and 25 mM potassium	NS	NS	NS
5 vs 25 mM potassium	NS	NS	NS
Amiben X N,P,K interactions			
H_1 vs H_2 X 0 vs 10 and 50 mM nitrate	NS	*	NS
H_1 vs H_2 X 10 vs 50 mM nitrate	*	NS	NS
H_1 vs H_2 X 0 vs 1 and 5 mM phosphate	NS	NS	NS
H_1 vs H_2 X 1 vs 5 mM phosphate	NS	NS	NS
H_1 vs H_2 X 0 vs 5 and 25 mM potassium	NS	NS	NS
H_1 vs H_2 X 5 vs 25 mM potassium	NS	NS	NS

*, **Significant at the 5 and 1% levels, respectively.

Table 2a. Atrazine-^{14}C absorption by corn, soybeans, and pigweed roots in 4 hr as influenced by nitrogen, phosphorus, and potassium levels in the nutrient solution

Atrazine absorption, dpm/mg root, dry wt									
	Corn			Soybeans			Pigweed		
Atrazine concn X 10^{-6} M	3.8	10	avg	3.8	10	avg	3.8	10	avg
Nitrate									
0	227	235	231	194	132	163	206	160	183
10 mM	246	244	245	202	187	194	162	154	158
50 mM	244	252	248	209	157	183	173	207	190
avg	239	244	242	202	159	180	180	174	177
Phosphate									
0	234	244	239	200	181	190	194	299	246
1 mM	240	225	232	202	187	194	230	193	212
5 mM	232	199	216	208	154	181	177	185	181
avg	235	223	229	203	174	188	200	226	213
Potassium									
0	221	229	225	184	187	186	185	226	206
5 mM	222	210	216	202	187	194	179	177	178
25 mM	203	265	234	183	159	171	177	210	194
avg	215	235	225	190	178	184	180	204	192
avg for N,P,K	230	234	232	198	170	184	187	201	194

Table 2b. Planned comparisons of atrazine and nutrient effects and their interactions on atrazine-^{14}C absorption by corn, soybean and pigweed roots in nutrient solutions

Comparison	Significance		
	Corn	Soybeans	Pigweed
Atrazine rate effect			
3.8×10^{-6} M (H_1) vs 10×10^{-6} (H_2) atrazine	NS	**	NS
N,P,K main effects			
0 vs 10 and 50 mM nitrate	NS	*	NS
10 vs 50 mM nitrate	NS	NS	NS
0 vs 1 and 5 mM phosphate	NS	NS	*
1 vs 5 mM phosphate	NS	NS	NS
0 vs 5 and 25 mM potassium	NS	NS	NS
5 vs 25 mM potassium	NS	NS	NS
Atrazine X N,P,K interactions			
H_1 vs H_2 X 0 vs 10 and 50 mM nitrate	NS	NS	NS
H_1 vs H_2 X 10 vs 50 mM nitrate	NS	NS	NS
H_1 vs H_2 X 0 vs 1 and 5 mM phosphate	NS	NS	**
H_1 vs H_2 X 1 vs 5 mM phosphate	NS	NS	NS
H_1 vs H_2 X 0 vs 5 and 25 mM potassium	NS	NS	NS
H_1 vs H_2 X 5 vs 25 mM potassium	*	NS	NS

*,**Significant at the 5 and 1% levels, respectively.

decrease in atrazine uptake by soybeans caused by 3.8 X 10^{-6} M atrazine when compared to the 10 X 10^{-6} M rate.

Nitrogen had no effect on linuron absorption (Table 3) by soybeans and pigweed. With corn there was a significant decrease in uptake between the 10 mM and 50 mM NO_3^- levels. for both herbicide rates. Higher N may have stimulated the overall physiological processes of the plant, but whether the plants responded to this during a 4 hr treatment is doubtful. The expected effect would be an increase in ion absorption and not a decrease. The results could be explained by competition for uptake sites. As the NO_3^- level increased from 10 to 50 mM, the absorption of linuron decreased because fewer carrier sites were available. This observation is also in agreement with Stein's (1967) statement that the flow of one substrate can stimulate the flow of another in the opposite direction.

Phosphorus had no effect on amiben absorption (Table 1) by corn, but its presence decreased amiben uptake significantly in soybeans. Comparing zero PO_4^{3-} to 1 and 5 mM PO_4^{3-} for pigweed, there were no significant differences. However, 1 mM PO_4^{3-} significantly decreased amiben uptake when compared to the uptake at 5 mM. The same trend was present in soybeans.

Atrazine uptake (Table 2) by corn and soybeans was unaffected by P, but the presence of P decreased atrazine uptake by pigweed. There was a significant interaction

Table 3a. Linuron-^{14}C absorption by corn, soybeans, and pigweed roots in 4 hr as influenced by nitrogen, phosphorus, and potassium levels in the nutrient solution

Linuron absorption, dpm/mg root, dry wt									
	Corn			Soybeans			Pigweed		
Linuron concn X 10^{-6} M	2.9	10	avg	2.9	10	avg	2.9	10	avg
Nitrate									
0	339	385	362	819	703	761	408	464	436
10 mM	403	389	396	729	685	707	566	508	537
50 mM	371	318	344	765	684	724	521	532	426
avg	371	364	368	771	691	731	498	501	500
Phosphate									
0	382	397	390	780	647	714	771	312	542
1 mM	368	378	373	829	679	754	484	509	496
5 mM	305	386	346	632	679	656	487	495	491
avg	352	387	369	747	668	708	581	439	510
Potassium									
0	395	383	389	812	687	750	484	472	478
5 mM	386	386	386	779	682	730	525	508	516
25 mM	391	390	390	890	712	801	497	553	525
avg	391	386	388	827	694	760	502	511	506
avg for N,P,K	371	379	375	782	684	733	527	484	506

Table 3b. Planned comparisons of linuron and nutrient effects and their interactions on linuron-^{14}C absorption by corn, soybean and pigweed roots in nutrient solutions

Comparison	Significance		
	Corn	Soybeans	Pigweed
Linuron rate effect			
2.9 X 10^{-6} M (H_1) vs 10 X 10^{-6} M (H_2) linuron	NS	**	NS
N,P,K main effects			
0 vs 10 and 50 mM nitrate	NS	NS	NS
10 vs 50 mM nitrate	*	NS	NS
0 vs 1 and 5 mM phosphate	NS	NS	NS
1 vs 5 mM phosphate	NS	**	NS
0 vs 5 and 25 mM potassium	NS	NS	NS
5 vs 25 mM potassium	NS	*	NS
Linuron X N,P,K interactions			
H_1 vs H_2 X 0 vs 10 and 50 mM nitrate	NS	NS	NS
H_1 vs H_2 X 10 vs 50 mM nitrate	NS	NS	NS
H_1 vs H_2 X 0 vs 1 and 5 mM phosphate	NS	NS	**
H_1 vs H_2 X 1 vs 5 mM phosphate	NS	**	NS
H_1 vs H_2 X 0 vs 5 and 25 mM potassium	NS	NS	NS
H_1 vs H_2 X 5 vs 25 mM potassium	NS	NS	NS

*,**Significant at the 5 and 1% levels, respectively.

with pigweed between the two atrazine concentrations and the 0 vs the 1 and 5 mM PO_4^{3-} levels.

Linuron uptake (Table 3) by corn was unaffected by P treatment. For the soybeans there was a highly significant interaction between the linuron concentration and the 1 and 5 mM PO_4^{3-} levels. With 2.9 X 10^{-6} M linuron, the 5 mM PO_4^{3-} rate reduced the uptake of linuron as compared to the 1 mM PO_4^{3-} treatment. With 10 X 10^{-6} M linuron in the solution, uptake was unaffected as the P level changed. This was opposite the response of the amiben and P level interaction. Thus the herbicide uptake responses to P seemed to vary with species, herbicide, and herbicide and P concentration. This was further illustrated in the case of linuron uptake by pigweed. There was a reversal in the P effect between the two linuron levels. At the zero PO_4^{3-} rate and 2.9 X 10^{-6} M linuron, absorption of linuron was increased by 1 and 5 mM PO_4^{3-}. Although, the uptake of linuron at the zero PO_4^{3-} level in 10 X 10^{-6} M linuron was greatly reduced when compared to the 1 and 5 mM PO_4^{3-} rates. The nature of the P effects on herbicide absorption is by no means clear.

Changes in the K concentration of the nutrient solution had no significant effects on amiben absorption at either level of amiben in any species (Table 1). There were no significant main effects of K on atrazine or linuron absorption by soybeans, corn or pigweed.

A consistent effect observed was the decreased amiben absorption in the presence of N by the susceptible species, corn and pigweed. It seemed important to study this further and to determine the influence of solution pH and the source of nitrogen on amiben uptake.

The experiment to determine the effects of N level and source showed highly significant interactions between the rates and sources of N and the nutrient solution pH for amiben-^{14}C uptake by corn roots (Figure VI). The orthogonal comparisons revealed highly significant differences between the nitrate and ammonium forms of N and these interacted with the highly significant linear pH effects (Table VI, Appendix). More amiben was taken up by corn with ammonium sulfate as the source of N than with potassium and calcium nitrate. No such interactions were noted in the case of amiben uptake by pigweed due perhaps to much more plant to plant variation in uptake.

The effect is due to the differences in the source of N since sulfate, potassium and calcium were present in both the nitrate and ammonium solutions. This effect was opposite to the one reported by McReynolds and Tweedy (1969) in which approximately twice as much simazine-^{14}C was translocated to the shoots of plants, grown in NO_3-N than in NH_4-N. Their plants were grown 4 days in solutions of simazine and therefore the differences probably reflect secondary effects and not differences in absorption alone.

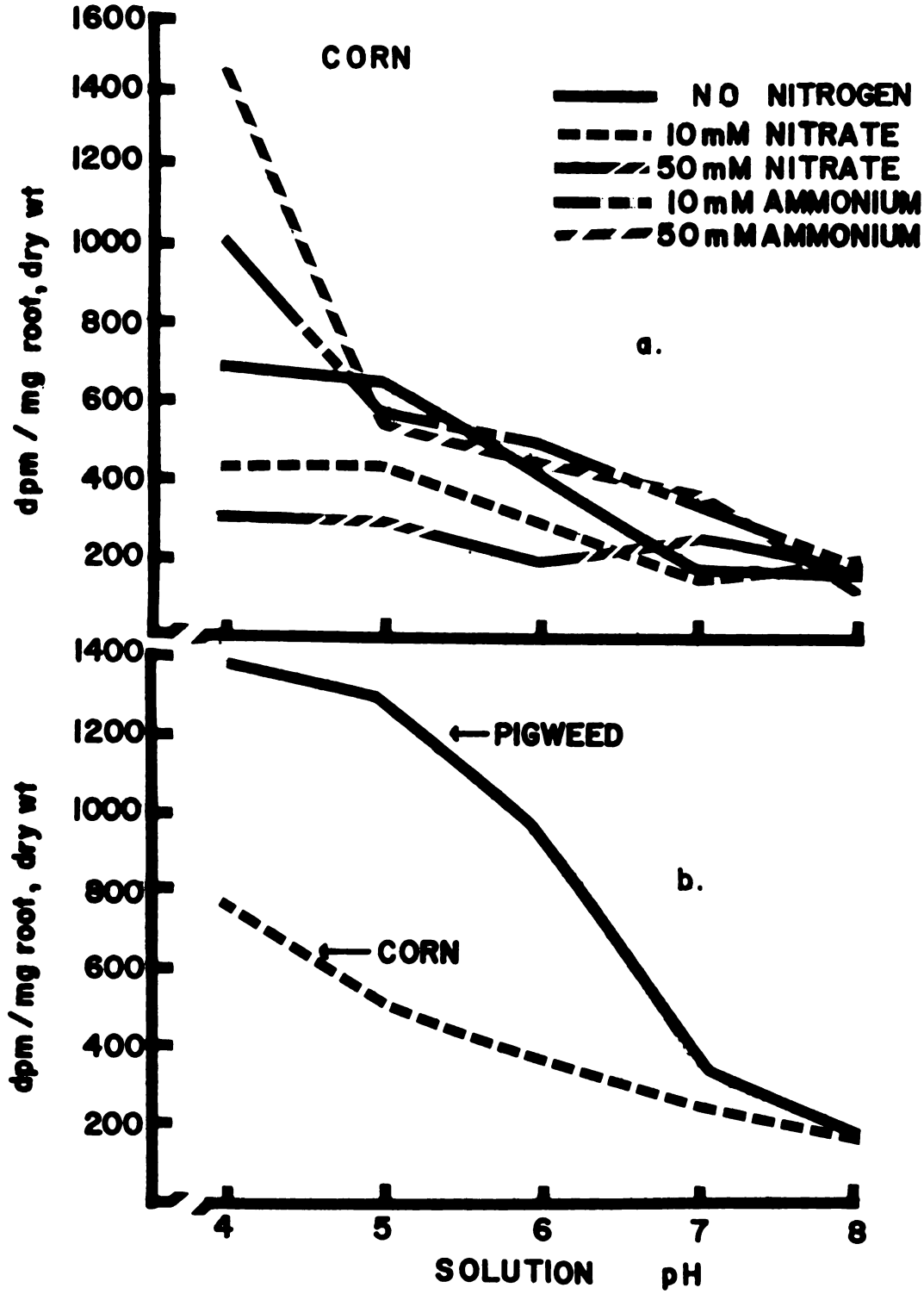

Figure VI. (a) Interaction of source and rate of nitrogen with solution pH on amiben-^{14}C uptake in 4 hr from nutrient solutions by corn roots
(b) The main effect of pH on amiben-^{14}C absorption in 4 hr by corn and pigweed roots from a nutrient solution

Since they used a different herbicide, no direct comparisons of results are justified. However, both amiben and simazine absorption and/or translocation seem to be affected by the source of N in the culture solution.

The interactions between N source and pH could be a reflection of the buffering capacity of the different solutions. If the NH_4-N source were a better buffer than the NO_3-N source, the high uptake at pH 4.0 for 10 and 50 mM NH_4^+ could be because the pH remained low. Perhaps in the 10 and 50 mM NO_3^- solutions the pH increased during the 4 hr period and the decreased uptake was a secondary effect. Determination of the buffering capacity of all solutions showed this did not occur (Table VII, Appendix). Each solution contained phosphate and was somewhat buffered. For a given pH the buffering capacities of the N solutions were relatively constant. Differential changes in solution pH then did not affect the results.

The linear effect of pH on amiben absorption was highly significant for both corn and pigweed (Figure VIb). These effects were determined by the orthogonal polynomial method where n=5 (Anderson and Houseman, 1942). Pigweed uptake of amiben was more affected by the solution pH than was corn uptake.

The amiben absorption response to pH changes could be due to any of three mechanisms. The first is simply competitive uptake between amiben ions and the hydroxyl

ions as proposed by Rains, et al. (1964). However this hypothesis has some practical limitations. It would seem quite unlikely that two molecules of such unequal size (MW amiben ion = 206, MW hydroxyl ion = 17) are taken up via the same pathway as competition is normally observed between similar sized molecules.

The form of the amiben molecule is important in determining its availability for absorption. Amiben can be expected to behave as an amino acid in solution since it has a carboxyl and amino moiety on the benzene ring. The degree of dissociation and charge on the molecule will change as pH changes. Since the pK_1 of amiben is 2.18, the pK_2 is 11.65, and the isoelectric point is 6.80 (Figure I, Appendix), the form of the ion changes from more positively charged ions at pH 4 to near neutrality at pH 7, to a greater number of negatively charged ions at pH 8. Amiben may be taken up as a positively charged ion and the linear decrease in absorption between pH 4 and 8 corresponds to the linear change in the titration curve for amiben in this pH range.

The increases in solution pH may have injured the plant roots. However, injury would be more likely to occur at the very low (pH 4) or high (pH 8) pH values relative to the normal soil solution pH of 5-7 for the maximum growth of most plants. Since the treatment time was only 4 hr, plant injury was not visible, yet the carrier system

for amiben could have been injured by the increasing hydroxyl ion concentration.

As the solution pH increased from 5 to 7, amiben absorption decreased 29.5 and 73.2% for corn and pigweed respectively. These pH values are found in the field and if the same effect on amiben absorption occurs there, it may explain a lack of good weed control at pH 7 or the presence of crop injury at pH 5.

A NO_3-N source reduced amiben uptake by corn roots while an NH_4-N source increased it. Perhaps the uptake of nitrate reduced amiben absorption according to the scheme of Stein mentioned earlier, i.e. an increase in the uptake of one substrate decreased the uptake of another. Nitrate uptake might increase while reducing amiben absorption. No explanation of the NH_4^+ effect is proposed.

Coleoptile absorption of amiben was measured in two ways, dpm/mm and dpm/mg coleoptile. Expressing the data as dpm/mg accounted for more variation than expressing it as dpm/mm. The surface area of the coleoptile is evidently more important in determining herbicide absorption than is the length. Coleoptile length and surface area are correlated, but the weight (which is a function of both length and diameter) provides a better estimate of surface area and therefore explains more of the treatment variation.

Amiben-^{14}C uptake by corn coleoptiles is both strikingly similar to and different than root absorption.

The effect of pH on amiben absorption by corn coleoptiles is very similar to the effect on root uptake. As pH increases, amiben uptake decreases and the analysis of variance shows that the linear effect of pH was highly significant while none of the higher order polynomials were significant. Increasing the pH from 5.0 to 7.0 decreased corn coleoptile absorption of amiben 30.5%. This is nearly identical to the 29.5% reduction in corn root absorption caused by the same pH change. This suggests that the same mechanism is operative in both root and shoot amiben uptake.

Nitrogen source and rate have no effect on amiben absorption by the coleoptile (Table 4). Since roots are the principal if not exclusive site of nutrient uptake from the soil, the lack of a nutrient effect on shoot uptake of a herbicide is logical.

It is important to compare the quantity of herbicide absorbed by the corn root system to that of the coleoptile. Expressing the overall means for the root and shoot uptake on an equal time basis showed the shoots and roots absorb 23.3 and 103.1 dpm/mg per hour respectively. Some caution must be used in this comparison as 5-day old plants were used in the root trials while 3-day old plants were used in the shoot absorption trials. The fact that the roots took up 443% more amiben than the shoots might be a reflection of the younger age and size of the coleoptiles. However, the coleoptiles were nearly as large

Table 4. Amiben-^{14}C absorption by corn coleoptiles in 90 min as influenced by solution pH and nitrogen source and level

pH of solution	dpm/mg coleoptile, dry wt	Nitrogen solution	dpm/mg coleoptile, dry wt
4.0	51.8	control	35.2
5.0	37.0	10mM NO_3^-	34.1
6.0	34.1	50mM NO_3^-	34.4
7.0	25.7	10mM NH_4^+	35.7
8.0	25.8	50mM NH_4^+	34.9

as they would become in field conditions and they had probably reached the stage of maximum herbicide absorption.

Even though corn coleoptiles appear to absorb much less amiben than the roots, the degree of phytotoxicity can not be determined from these experiments. The concentration of amiben absorbed by the coleoptiles may cause great phytotoxic effects. Stoller and Wax (1968) report relatively little upward translocation of amiben in morning glory (Ipomoea hederacea L.), velvet leaf (Abutilon theophrasti Medic.), white beans, barley (Hordeum vulgare L.), carrots, tomatoes, and sugar beets (Beta vulgaris L.). Translocation differences did not explain the selectivity of amiben. Since little amiben is translocated, its mode of action may occur in the roots. Then phytotoxicity would not depend on translocation to the shoot and this might

explain why Knake (1968) found shoot uptake of amiben by giant foxtail less phytotoxic than root uptake. Variation in uptake from species to species and chemical to chemical make specific statements concerning shoot uptake at this point impossible, although shoot uptake is an important process in the expression of phytotoxicity for many chemicals in several species (Knake, 1967, 1968; Appleby and Furtick, 1965; and Appleby et al. 1965).

Amiben phytotoxicity was studied in a bioassay experiment with two sources of nitrogen, NO_3^- and NH_4^+, since the first two bioassays used only NO_3^-. Phytotoxicity increased when NH_4^+ was the N source (Table 5). With 8 ppm amiben the injury ratings and percent shoot reductions of oats were significantly increased by 10 and 50 mM NH_4^+. In this experiment N-serve was added to the NO_3^- solutions to prevent bacterial conversion of NO_3^- to NH_4^+. The rate of .1 ml N-serve/mM NO_3^- per liter solution was itself phytotoxic to the oats. At 50 mM NO_3^-, the oats were completely killed by N-serve and the effect of amiben at various solution pH's with NO_3^- could not be determined.

It was shown that amiben phytotoxicity increased with both NO_3^- and NH_4^+ while amiben absorption varied with these same treatments. Therefore, the increased phytotoxicity observed in the bioassay experiments was not caused by increased amiben absorption in NO_3^- solutions.

Table 5. Effect of pH and ammonium level on the phytotoxicity of 8 ppm amiben to oats grown in sand culture

N level	Solution pH	% shoot reduction	Injury rating[1]
0	4.0	63.1	4.5
0	5.0	63.0	3.5
0	6.0	53.0	4.0
0	7.0	47.1	4.5
0	8.0	53.1	3.5
avg		55.9	4.0
10 mM NH_4^+	4.0	74.2	7.0
10 mM NH_4^+	5.0	67.6	6.5
10 mM NH_4^+	6.0	70.8	6.5
10 mM NH_4^+	7.0	70.1	6.0
10 mM NH_4^+	8.0	66.5	4.0
avg		69.8	6.0
50 mM NH_4^+	4.0	68.9	8.5
50 mM NH_4^+	5.0	70.1	8.5
50 mM NH_4^+	6.0	77.8	8.0
50 mM NH_4^+	7.0	76.8	8.0
50 mM NH_4^+	8.0	78.3	8.5
avg		74.4	8.3

[1]0 = no injury, 10 = complete kill.

PLANNED COMPARISON	SIGNIFICANCE	
	% Shoot Reduction	Injury Rating
Ammonium effects		
0 N vs 10 and 50 mM NH_4^+	**	**
10 vs 50 mM NH_4^+	NS	**
pH effects		
pH linear	NS	*
pH quadratic	NS	NS
pH cubic	NS	NS
pH quardic	NS	NS
Ammonium and pH interactions[2]		
10 vs 50 mM NH_4^+ X pH linear	NS	**

[2]Only significant interactions are given.

*,**Significant at the 5 and 1% levels respectively.

SUMMARY AND CONCLUSIONS

Bioassay experiments with oats grown 3 weeks in sand culture were performed to determine herbicide and nutrient interactions with herbicide toxicity. They showed that the toxicity of amiben, atrazine, and linuron increased as the NO_3^- level of the nutrient solution increased. Phosphorus and potassium did not interact with herbicide toxicity. Amiben toxicity was increased by both NO_3^- and NH_4^+ sources of N. Thus the application of higher rates of fertilizer should have no negative effects on herbicide effectiveness and with higher N levels the toxicity may even increase.

To determine if the increased toxicity was due to greater herbicide uptake, root absorption experiments investigated possible interactions on the uptake of ^{14}-C-herbicides with nutrients. There were few significant interactions between atrazine or linuron absorption and the nutrient level. An important response was that NO_3^- decreased amiben absorption by corn and pigweed roots.

This indicates that amiben's increased toxicity at higher NO_3^- rates in the bioassay experiments was not due to increased amiben uptake. Since the nitrogen

level did not increase atrazine or linuron uptake, their greater toxicity with increased N rate also was not at the site of uptake.

The effects of different N sources and nutrient solution pH's on amiben uptake by corn and pigweed roots were investigated. An NH_4^+ nitrogen source compared to a NO_3^- source increased amiben uptake in corn but not in pigweed. Amiben absorption decreased linearly between pH 4.0 and 8.0. There were highly significant interactions between N source and solution pH such that the differences in amiben uptake between NO_3-N and NH_4-N were greater at low pH values.

These effects might have practical aspects as nitrogen fertilization of soybeans becomes more common. If soybeans and weed species in the field respond to the pH influence on amiben uptake like corn grown in nutrient culture, the form of N fertilizer added when amiben is applied could greatly influence both weed control and crop injury. When ammonium sulfate is the N carrier, the danger of soybean injury might increase; whereas, with potassium nitrate as the source of N, injury would be less likely although weed control might be less than ideal. Therefore, in addition to the many factors one must already consider in applying herbicides, the form of nitrogen fertilizer applied may also be important.

The effects of N source and solution pH on amiben-^{14}C absorption by intact corn coleoptiles was studied since shoot uptake of soil applied herbicides is important. A technique was developed to treat only intact corn coleoptiles.

There was a linear decrease of amiben absorption as pH values increased from 4.0 to 8.0. Between these values amiben changes from a positively charged ion to a negatively charged ion which suggests that amiben is absorbed as a positively charged ion. The absorption mechanisms of coleoptiles and roots may be the same since their response to pH change is identical.

Corn roots absorbed 443% more amiben than the coleoptiles on a mg basis, which suggests that the root may be the main site of amiben entry in corn.

LITERATURE CITED

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1. Adams, R. S., Jr. 1964. Fertilizer placement critical where herbicide residues are present. Minn. Farm and Home Sci. 22: 13-14.

2. _____. 1965. Phosphorus fertilization and the phytotoxicity of simazine. Weeds: 113-116.

3. Anderson, R. L. and E. E. Houseman. 1942. Tables of orthogonal polynomial values extended to N=104. Iowa State Res. Bull. 297: 595-672.

4. Appleby, A. P. and W. R. Furtick. 1965. A technique for controlled exposure of emerging grass seedlings to soil-active herbicides. Weeds 13: 172-173.

5. _____, _____, and S. C. Fang. 1965. Soil placement studies with EPTC and other carbamate herbicides on Avena sativa. Weed Res. 5: 115-122.

6. Bingham, S. W. and R. P. Upchurch. 1959. Some interactions between nutrient level (N,P,K,Ca) and diuron in the growth of cotton and Italian ryegrass. Weeds 7: 167-177.

7. Broyer, T. C. 1956. Current views on solute movement into plant roots. Proc. Amer. Soc. Hort. Sci. 67: 570-586.

8. Cooke, A. R. 1956. A possible mechanism of action of the urea type herbicides. Weeds 4: 397-398.

9. _____. 1957. Influence of 2,4-D on the uptake of minerals from the soil. Weeds 5: 25-28.

10. Corbin, F. T. and R. P. Upchurch. 1967. Influence of pH on detoxification of herbicides in the soil. Weeds 15: 370-377.

11. Crafts, A. S. 1939. The relation of nutrient to toxicity of arsenic, borax and chlorate in soils. J. Agr. Res. 58: 637-671.

12. _____. 1959. Further studies on comparative mobility of labeled herbicides. Plant Physiol. 34: 613-620.

13. _____. 1964. Herbicide behavior in the plant, p. 75-110. In L. J. Audus (ed.) The physiology and biochemistry of herbicides. Academic Press, New York.

14. Dhillon, P. S., W. R. Byrnes and C. Merritt. 1967. Simazine and phosphorus interactions in red pine seedlings. Weeds 15: 339-343.

15. Donaldson, T. W. 1967. Absorption of herbicides 2,4-D and monuron by barley roots. Diss. Abstr. 28: 1371-B.

16. Elzam, O. E. and E. Epstein. 1965. Absorption of chloride by barley roots: kinetics and selectivity. Plant Physiol. 40: 620-624.

17. Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36: 437-444.

18. Fang, S. C. and J. S. Butts. 1954. Studies in plant metabolism. IV. Comparative effects of 2,4-D and other plant growth regulators on phosphorus metabolism in bean plants. Plant Physiol. 29: 365-368.

19. Fried, M. and J. C. Noggle. 1958. Multiple site uptake of individual cations by roots as affected by hydrogen ion. Plant Physiol. 33: 139-144.

20. _____, and R. E. Shapiro. 1961. Soil-plant relationships in ion uptake. Annu. Rev. Plant Physiol. 12: 91-112.

21. Herberg, R. J. 1965. Channels ratio method of quench correction in liquid scintillation counting. Packard Tech. Bull. 15. 8p.

22. Hogue, E. J. 1968. The effect of linuron on ^{32}P and ^{45}Ca uptake in tomato and parsnip. Weed Sci. 16: 185-187.

23. Jennings, O. H. 1963. The absorption of solutes by plant cells. Iowa State Univ. Press, Ames. 204 p.

24. Knake, E. L., A. P. Appleby and W. R. Furtick. 1967. Soil incorporation and site of uptake of preemergence herbicides. Weeds 15: 228-232.

25. _____, and L. M. Wax. 1968. The importance of the shoot of giant foxtail for uptake of preemergence herbicides. Weed Sci. 16: 393-395.

26. Loustalot, A. J., M P. Morris, J. Garcia and C. Pagan. 1953. 2,4-D effects on phosphorus metabolism. Sci. 118: 627-628.

27. McReynolds, W. D. Jr., and J. A. Tweedy. 1969. Effect of nitrogen form on the uptake and herbicidal activity of simazine in corn, rye and soybeans. Abstr. Weed Sci. Soc. Amer. 1969 Meeting. No. 213.

28. Moreland, D. E., W. A. Gentner, J. L. Hilton and K. L. Hill. 1959. Studies on the mechanism of herbicidal action of 2-chloro-4,6-bis(ethylamino)-s-triazine. Plant Physiol. 34: 432-435.

29. Nash, R. G. 1968. Plant uptake of ^{14}C-diuron in modified soil. Agron. J. 60: 177-179.

30. Nashed, R. B. and R. O. Ilnicki. 1968. The effect of linuron on ion uptake in corn, soybeans, and crabgrass. Weed Sci. 16: 188-192.

31. Oliver, L. R. and R. E. Frans. 1968. Inhibition of cotton and soybean roots from incorporated trifluralin and persistence in soil. Weed Sci. 16: 199-203.

32. Pardee, A. B. 1968. Membrane transport proteins. Sci. 162: 632-637.

33. Rains, D. W., W. E. Schmid and E. Epstein. 1964. Absorption of cations by roots. Effects of hydrogen ions and essential role of calcium. Plant Physiol. 39: 274-278.

34. Rebstock, T. L., C. L. Hamner and H. M. Sell. 1954. The influence of 2,4-D on the phosphorus metabolism of cranberry bean plants. (Phaseolus vulgaris). Plant Physiol. 29: 490-491.

35. Ries, S. K., R. P. Larsen and A. L. Kenworthy. 1963. The apparent influence of simazine on nitrogen nutrition of peach and apple trees. Weeds 11: 270-273.

36. _____, and A. Gast. 1965. The effects of simazine on nitrogenous components of corn. Weeds 13: 272-273.

37. Rumberg, C. B., R. E. Engel and W. F. Meggitt. 1960. Effect of phosphorus concentration on the absorption of arsenate by oats from nutrient solutions. Agron. J. 52: 452-453.

38. Sabbe, W. E. 1967. Effect of trifluralin on uptake of fertilizer phosphorus. Ark. Farm Res. 16: 4.

39. Stein, W. D. 1967. The movement of molecules across cell membranes. Academic Press, New York. 369 p.

40. Stoller, E. W. and L. M. Wax. 1968. Amiben metabolism and selectivity. Weed Sci. 16: 283-288.

41. Sutcliffe, J. F. 1962. Mineral salts absorption in plants. Pergamon Press, New York. 44 p.

42. Upchurch, R. P., G. R. Ledbetter and F. L. Selman. 1963. The interaction of phosphorus with the phytotoxicity of soil applied herbicides. Weeds 11: 36-41.

43. _____, F. L. Selman, D. D. Mason and E. J. Kamprath. 1966. The correlation of herbicidal activity with soil and climate factors. Weeds 14: 42-49.

44. Vega, M. P. 1964. The influence of phosphate on the phytotoxicity of 3-amino-1,2,4-triazole. Diss. Abstr. 25: 82.

45. Wang, C. H. and D. L. Willis. 1965. Radiotracer methodology in biological science. Prentice-Hall, Inc., N. J. 363 p.

46. Weber, J. B., P. W. Perry and K. Ibaraki. 1968. Effect of pH on the phytotoxicity of prometryne applied to synthetic soil media. Weed Sci. 16: 134-136.

47. Wort, D. J. 1962. The application of sublethal concentrations of 2,4-D in combination with mineral nutrients. World Rev. of Pest Control 1: 6-19.

APPENDIX

Table I. Phytotoxicity of three levels of amiben to oats grown in a sand culture with various nutrient solutions

Nutrient Solution	Percent reduction in shoot dry wt			
	Amiben concn, ppm			
	4	8	16	avg
NPK[1]	53	59	75	62
-N[2]	29	50	64	47
-P[2]	41	66	69	59
-K[2]	57	55	74	62
avg	45	58	70	58

[1]Full strength Hoagland's # 1 nutrient solution.

[2]Full strength Hoagland's for all other essential elements.

COMPARISON	SUM of SQUARES
Amiben effects	
Amiben, linear	3909**
Amiben, quadradic	2
Nutrient effects[3]	
NPK vs -N	956**
NPK vs -P	53
NPK vs -K	0
Amiben X nutrient interactions	
Linear X NPK vs -N	120*
Linear X NPK vs -P	24
Linear X NPK vs -K	22
Quadradic X NPK vs -N	61
Quadradic X NPK vs -P	254**
Quadradic X NPK vs -K	28

*,**Significant at the 5 and 1% levels respectively.

[3]Nonorthogonal comparisons.

Table II. Phytotoxicity of three levels of atrazine to oats grown in a sand culture with various nutrient solutions

Nutrient Solution	Percent reduction in shoot dry wt			
	Amiben concn, ppm			
	4	8	16	avg
NPK[1]	84	84	83	84
-N[2]	60	64	63	62
-P[2]	81	81	83	82
-K[2]	68	78	81	76
avg	73	77	78	76

[1]Full strength Hoagland's # 1 nutrient solution.

[2]Full strength Hoagland's for all other essential elements.

COMPARISON	SUM of SQUARES
Atrazine effects	
Atrazine, linear	99*
Atrazine, quadradic	13
Nutrient effects[3]	
NPK vs -N	2056**
NPK vs -P	21
NPK vs -K	308**
Atrazine X nutrient interactions	
Linear X NPK vs -N	16
Linear X NPK vs -P	10
Linear X NPK vs -K	156*
Quadradic X NPK vs -N	0
Quadradic X NPK vs -P	2
Quadradic X NPK vs -K	4

*,**Significant at the 5 and 1% levels respectively.

[3]Nonorthogonal comparisons.

Table III. Amiben phytotoxicity to oats grown three weeks in sand culture at three levels of nitrate as measured by oat shoot injury ratings and dry weights

	Injury rating[1]				Dry wt, mg/10 shoots			
mM Nitrate	Amiben, ppm				Amiben, ppm			
	0	4	8	avg	0	4	8	avg
0	2.0	3.6	4.5	3.4	165	192	165	174
5	0.1	2.9	4.3	2.4	292	209	177	226
10	0.5	2.6	3.8	2.3	363	228	192	261
avg	0.9	3.0	4.2	2.7	273	210	178	220

Planned Comparisons	Significance Injury	Dry wt
<u>Amiben effects</u>		
0 vs 4 and 8 ppm amiben	**	**
4 vs 8 ppm amiben	**	**
<u>Nitrogen effects</u>		
0 vs 5 and 10 mM nitrate	**	**
5 vs 10 mM nitrate	NS	**
<u>Amiben X nitrate interactions</u>		
0 vs 4 and 8 ppm amiben X 0 vs 5 and 10 mM NO_3^-	**	**
4 vs 8 ppm amiben X 0 vs 5 and 10 mM NO_3^-	NS	NS
0 vs 4 and 8 ppm amiben X 5 vs 10 mM NO_3^-	*	**
4 vs 8 ppm amiben X 5 vs 10 mM NO_3^-	NS	NS

[1] 0 = no injury, 10 = complete kill.

*,**Significance at the 5 and 1% level respectively.

Table IV. Atrazine phytotoxicity to oats grown three weeks in sand culture at three levels of nitrate as measured by oat shoot injury ratings and dry weights

	Injury rating[1]				Dry wt, mg/10 shoots			
mM Nitrate	Atrazine, ppm				Atrazine, ppm			
	0	1	2	avg	0	1	2	avg
0	1.4	5.7	7.6	4.9	136	64	61	87
5	0.0	9.0	9.9	6.3	245	62	62	123
10	0.0	9.3	9.7	6.3	317	80	61	153
avg	0.5	8.0	9.0	5.8	233	68	62	121

Planned Comparisons	Significance: Injury	Significance: Dry wt
Atrazine effects		
0 vs 1 and 2 ppm atrazine	**	**
1 vs 2 ppm atrazine	**	NS
Nitrate effects		
0 vs 5 and 10 mM nitrate	**	**
5 vs 10 mM nitrate	NS	**
Atrazine X nitrogen interactions		
0 vs 1 and 2 ppm atr X 0 vs 5 and 10 mM NO_3^-	**	**
1 vs 2 ppm atr X 0 vs 5 and 10 mM NO_3^-	**	NS
0 vs 1 and 2 ppm atr X 5 vs 10 mM NO_3^-	NS	**
1 vs 2 ppm atr X 5 vs 10 mM NO_3^-	NS	NS

[1] 0 = no injury, 10 = complete kill.

*,**Significant at the 5 and 1% levels respectively.

Table V. Linuron phytotoxicity to oats grown three weeks in sand culture at three levels of nitrate as measured by oat shoot injury ratings and dry weights

	Injury rating[1]				Dry wt, mg/10 shoots			
mM Nitrate	Linuron, ppm				Linuron, ppm			
	0	1	2	avg	0	1	2	avg
0	1.1	3.0	4.3	2.8	168	74	70	104
5	0.1	4.6	6.8	3.9	247	73	71	131
10	0.1	5.3	7.4	4.3	299	75	64	146
avg	0.4	4.3	6.2	3.6	238	74	68	127

Planned Comparisons	Significance: Injury	Significance: Dry wt
Linuron effects		
0 vs 1 and 2 ppm linuron	**	**
1 vs 2 ppm linuron	**	NS
Nitrogen effects		
0 vs 5 and 10 mM nitrate	**	**
5 vs 10 mM nitrate	*	NS
Linuron X nitrogen interactions		
0 vs 1 and 2 ppm lin X 0 vs 5 and 10 mM NO_3^-	**	**
1 vs 2 ppm lin X 0 vs 5 and 10 mM NO_3^-	*	NS
0 vs 1 ppm lin X 5 vs 10 mM NO_3^-	*	*
1 vs 2 ppm lin X 5 vs 10 mM NO_3^-	NS	NS

[1] 0 = no injury, 10 = complete kill.

*,**Significant at the 5 and 1% levels respectively.

Table VI. Influence of source and rate of nitrogen and the nutrient solution pH on amiben^{14}-C absorption, dpm/mg root, dry wt

Solution pH	Nitrogen source and rate					
	Control	Nitrate		Ammonium		
	-	10 mM	50 mM	10mM	50 mM	avg
4.0	687	423	310	1012	1424	771
5.0	651	438	304	578	540	502
6.0	400	288	195	495	452	366
7.0	172	168	243	324	362	254
8.0	172	198	185	179	110	169
avg	416	302	248	518	578	412

PLANNED COMPARISONS	SIGNIFICANCE
Nitrogen effects	
Control vs nitrogen	NS
Nitrate vs ammonium	**
10 mM NO_3^- vs 50 mM NO_3^-	NS
10 mM NH_4^+ vs 50 mM NH_4^+	NS
ph effects	
Linear	**
Quadradic	**
Cubic	NS
Nitrogen X pH interactions[1]	
NO_3^- vs NH_4^+ X pH linear	**
10 vs 50 mM NH_4^+ X pH linear	**
NO_3^- vs NH_4^+ X pH quadradic	**
10 vs 50 mM NH_4^+ X pH quadradic	*

[1]Only the significant interactions are given.

*,**=Significant at the 5 and 1% levels respectively.

Table VII. Buffering capacity of nutrient solutions solutions with different sources and rates of nitrogen

ml acid or base to adjust pH/100 ml nutrient solution						
pH adjusted from:	to:	Nitrogen source and rate[1]				
		Control	10 mM NO_3^-	50 mM NO_3^-	10 mM NH_4^+	50 mM NH_4^+
4.0	7.0[2]	1.94	2.23	2.97	2.23	2.57
5.0	7.0[2]	1.46	1.70	2.73	1.59	2.00
6.0	8.0[2]	2.85	3.64	3.88	3.09	6.06
7.0	5.0[3]	1.82	1.67	3.64	1.77	2.34
8.0	6.0[3]	3.18	3.42	2.32	2.62	5.08

[1]Solutions were complete for all other essential elements.

[2]Adjusted with .05 N NaOH.

[3]Adjusted with .05 H_2SO_4.

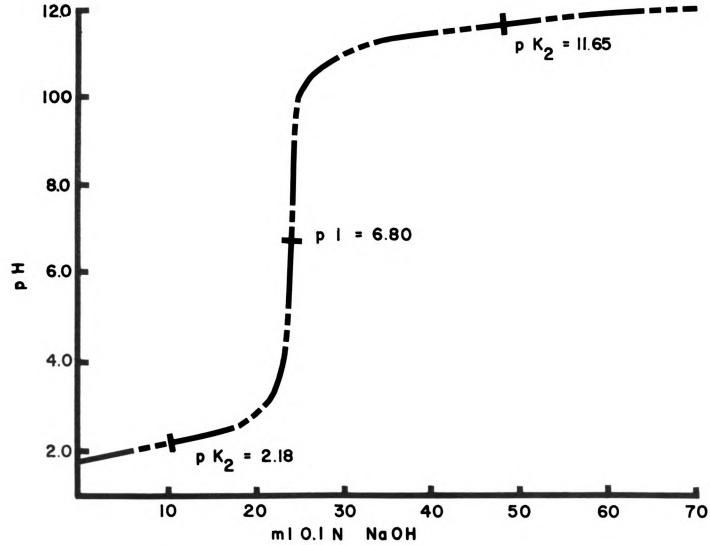

Figure 1. Titration curve for 2 X 10^{-3} M amiben titrated with 0.1 N NaOH